CORTADERIA JUBATA (GRAMINEAE)

AN AUTONOMOUS APOMICT

A thesis

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by

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Fig. 1

Cortaderia jubata flowering in the plots at Botany Division, D.S.I.R., Lincoln -- February, 1976.

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ABSTRACT

The literature relating to apomixis in members of the Gramineae is reviewed, particularly as it relates to the types of mechanisms found and to the occurrence of apomixis in the different systematic divisions of the family. Apomixis is more prevalent in the Subfamily Panicoideae than in the Pooideae, and the course of development of the unreduced embryo sac is distinctive in the two subfamilies. Throughout the grasses somatic apospory predominates over gonial apospory, and pseudogamy is much more prevalent than non-pseudogamy.

In *Cortaderia jubata* the spore mother cell (or its derivatives) degenerate and nucellar cells develop as somatic embryo sacs. These follow a course of development similar to the Hieracium type. The synergid develops a prominent haustorium which usually protrudes beyond the micropyle. No functional pollen forms and embryogenesis begins well before anthesis. The species is, therefore, non-pseudogamous. Since no other non-pseudogamous grass also exhibits somatic apospory the situation in *Cortaderia* would appear to be unique among apomictic grasses.

The sexual species of this genus are gynodioecious and exhibit a Polygonum type, 8-nucleate, 7-celled embryo sac, in which the polar nuclei fuse at or near fertilization and the two synergids develop haustorial extensions through the micropyle during the organisation of the megagametophyte.

The structure of the component cells of the somatic

embryo sac is described at the ultrastructural level and compared with published accounts. The progress of nucellar degeneration is discussed, and its association with dilatations of the nuclear envelope is noted. A deeply lobed nucleus which is intimately associated with both the apomictic egg and the synergid is characteristic of the central cell. Wall ingrowths of transfer-cell type appear during the organization of the somatic embryo sac and occur all over the central cell, except adjacent to the antipodal cells. Sexual species of *Cortaderia* also show this feature prior to fertilization. The structure of the haustorium and synergid is described and their role in nutrient uptake and transport is discussed in the light of their morphology. Antipodal cells appear also to be adapted to a nutritional function. The formation of walls between the coenocytic endosperm cells follows the pattern already described for this tissue, but wall formation between the cells of the megagametophyte follows a distinctive course. A preliminary account is given of the ultrastructure of the cells of the young globular embryo and the unusual presence of layers of dilated endoplasmic reticulum cisternae in the suspensor is recorded. Finally, notes are given on some miscellaneous topics revealed by the electron microscope.

INTRODUCTION

Breeding experiments by H.E. Connor (1974) have shown that several species of South American pampas grasses *(Cortaderia)*, including *C. jubata* (Lem.) Stapf, are obligate apomicts. No functional pollen is produced in the abortive stamens of this species.

The purpose of this investigation is to determine the type of apomixis involved in *Cortaderia jubata* as illustrated by the sequence of events leading up to embryo formation. These events are then compared with the apomictic mechanisms developed in other genera of the Gramineae. A number of the more striking cytological features were examined by transmission electron microscopy.

The genus *Cortaderia* comprises about twenty species distributed mainly in South America and New Zealand. Together with a few other genera, including *Arundo* and *Phragmites*, they form the Tribe Arundineae.[1] The characteristic facies of the spikelets of this Tribe result from the numerous florets with long, tapering glumes and the copious, long, silky hairs borne on the rhachilla or the dorsal face of the lemmata. The Tribe takes a position somewhat intermediate between the two main subfamilies — Pooideae (or Festucoideae) and Panicoideae — a situation recognized by Avdulov (1931), who subdivided the Festucoid grasses into

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[1] The Subfamilies and Tribes proposed by Hubbard (1959) are adopted. Zotov (1963) placed *Cortaderia* in a Tribe of its own (Cortaderieae) and modified the limits of the Arundineae, adopting Tateoka's (1957) treatment of this Tribe as a Subfamily (Arundoideae).

Festuciformes and Phragmitiformes. The Arundineae (with *Cortaderia*) fall into the latter group (see de Wet, 1954; Reeder, 1957).

A considerable number of reviews have appeared covering the mechanism, origin and evolutionary significance of apomixis. The most important of these (Darlington, 1937; Stebbins, 1941, 1950; Gustafsson, 1946-7; Nygren, 1954; 1966, 1967; Battaglia, 1963; Rutishauser, 1967; Grant, 1971) discuss, among other aspects of apomixis, the cytological breakdown of meiosis, the development of unreduced embryo sacs, the control of these phenomena by genetic and environmental factors, their relationship to polyploidy and hybridity, and their consequences in evolution, biosystematics and plant breeding. The phenomena are so complex and can be viewed from so many different aspects, that an inevitable conflict of terminology has arisen, which has not yet finally been resolved, since original schemes are still being proposed (Solntzeva, 1969). The present account will be limited to forms of apomixis in which a seed is developed, that is, to agamospermy. In addition, only those forms of agamospermy in which the embryo arises within an embryo sac will be considered, adventitious embryony being excluded (Maheshwari and Sachar, 1963). Within the limits set, at least two terminologies are current. That of Gustafsson (1946), adopted by Stebbins in his later treatment (1950), refers to the two main categories as apospory (origin of embryo sac from a somatic cell) and diplospory (origin from an unreduced megaspore mother cell or its derivatives. These two conditions are termed somatic apospory and gonial apospory respectively by Battaglia (1963)

and this usage is adopted by Davis (1966) in her systematic survey of embryology. Objections can be made to both sets of terms. Diplospory, which implies unreduced spores, appears inapplicable to embryo sacs developed directly from an undivided megaspore mother cell. On the other hand, gonial apospory, which implies that no spores are formed, appears inappropriate in those cases where forms of meiosis progress to some stage. However the latter two terms seem more straightforward and will be adopted in the present account.

No comprehensive review of apomixis as found within the Gramineae has appeared. Valuable sections of the reviews of Myers (1947), Nygren (1954) and Fryxell (1957) are devoted to apomixis in grasses, but these are all out of date. The considerable literature on apomixis in grasses has been searched, but this review will consider mainly works in which the embryological details of what Gustafsson called the mechanism of apomixis are described.

Although the Gramineae are one of the few families in which both somatic and gonial apospory occur, and Hanson (1972) could say "Both facultative and obligate forms of apomixis are relatively common in grasses", it is interesting to note that apomixis is not mentioned in Arber's "The Gramineae" (1934). In fact, it is since that time that virtually the whole of our knowledge of apomixis in the family has been acquired. Agamospermy is often said to be not infrequent among grasses, but in comparison to the size of the family its incidence is slight. Apomixis is reported in one or a few species belonging to approximately thirty-two genera in eleven of the twenty-five Tribes

recognised by Hubbard (1959). The lists published by Gustafsson (1946-7), Nygren (1954, 1967), Fryxwell (1957), Emery (1957), Brown (1958) and Brown and Emery (1958) have been brought up to date to include reports in many scattered sources. Genera which are not cited as apomictic by the above authors are listed below, with the authority for the occurrence of agamospermy.

TABLE 1

Recently Reported Agamospermous Grass Genera

Genus	Tribe	Authorities
Ischaemum	Andropogoneae	Oke, 1973
Sehima	"	"
Apluda	"	Murty, 1973
Cortaderia	Arundineae	Connor, 1974
Lamprothyrsus	Undetermined	Connor, 1976 (pers. comm.)

This list is not intended to be exhaustive, as it does not include cases of rare parthenocarpic development in grasses with otherwise normal sexual reproduction (e.g., *Buchloë* has a slight apomictic tendency (Brown and Emery, 1958), and among 1112 induced tetraploid *Coix* plants one diploid arose by parthenocarpy (Venkateswarlu and Rao, 1975)) nor does it include the occurrence of twin embryos recorded sporadically in several cereals (e.g., Sharman, 1942; Nielson, 1946). In most reports of this kind the origin of the twins is not established, but stimulation of an unfertilized egg by a neighbouring fertilized egg is sometimes postulated as a likely possibility.

The incidence of apomixis among the Tribes of the

Gramineae is decidedly uneven. It is only in the three Tribes of Subfamily Panicoideae that apomixis is rather prevalent (twelve genera of Andropogoneae, ten genera of Paniceae, and one genus of Maydeae). On the basis of this widespread occurrence of a characteristic form of apomixis in Panicoid grasses, Brown and Emery (1958) and Brown (1959) have postulated that the genes for this mechanism have been carried within the group since the Cretaceous and before generic limits, as we know them, were defined. In the Subfamily Pooideae the only Tribe with apomixis recorded in more than one genus is the Chlorideae (with *Chloris*, *Bouteloua* and *Fingerhoutia*), and few genera have several species known to be apomictic (e.g., *Poa*, *Calamagrostis*, *Hierochloë*). Thorough surveys are needed, but since several of these Tribes are relatively well-known, it must be accepted that apomixis is not prevalent in the Festucoid grasses.

Not only is apomixis relatively more frequent within the Panicoideae, but it displays distinctive morphological characteristics in that Subfamily. The somatic (nucellar) embryo sacs characteristically undergo their first division before vacuolation of the cytoplasm is effected, the two daughter nuclei remain together at one end of the sac and divide only once to give a mature four-nucleate sac with (normally) two synergids, an egg and one polar nucleus. This embryo sac is similar to the Oenothera Type, but is, of course, unreduced. This type of aposporous unreduced embryo sac has been called the Panicum Type (Battaglia, 1963; Rutishauser, 1967). The embryo sacs found in Festucoid apomicts are very varied but relate to the Polygonum Type which is characteristic of the sexual embryo

sacs of all grasses, including the Panicoid genera. This sequence of apomictic unreduced embryo sac development has been termed the Hieracium Type.

The main features of the agamospermous apomictic mechanisms of grasses are well documented. Those to which most attention has been directed are the origin of the embryo sacs and the role of pollination in the stimulation of embryo and endosperm development. The information contained in a number of reports is summarized in Table 2. This list contains only a selection from the available accounts, but those chosen are representative of the systematic range of apomixis, and mostly stress the embryological approach. This list is intended merely as an indication of the broad situation which of necessity is simplified. There are several reasons for this. Primarily, the mechanism of apomixis is rarely rigidly determined, for in many species it consists of a balance between several pathways. Secondly, the information available may be incomplete or conflicting.

It is evident from Table 2 that apomictic grasses are preponderantly pseudogamous. The only grasses other than *Cortaderia* known to me to be non-pseudogamous are *Poa nervosa* (Grun, 1955), *Nardus stricta* (Rychlewski, 1961) and *Calamagrostis* species (Nygren, 1946, 1954). In *Calamagrostis chalybaea* functional pollen is not produced and in *C. purpurea* it is very rare, while in *C. lapponica* pollen is generally functional. However, in all these species pollination is not required for development of the apomictic embryo or endosperm. In *Poa nervosa* pollen is entirely non-functional over most of its range. However, unlike *Cortaderia jubata*,

TABLE 2

Types of Apomictic Mechanisms in Grasses

A. PSEUDOGAMOUS

I. SPECIES WITH SOMATIC APOSPORY
(Gonial apospory and amphimixis may also occur)

Agropyron scabrum	Hair, 1956
Apluda mutica	Murty, 1973
Bothriochloa intermedia	Saran and de Wet, 1970
" *ischaemum*	Brown and Emery, 1957
" *pertusa*	Gupta, 1969-70
Bouteloua curtipendula	Mohamed and Gould, 1966
Cenchrus setigerus	Fisher *et al.*, 1954; Snyder *et al.*, 1955
Dichanthium annulatum	Reddy and d'Cruz, 1969
Heteropogon contortus	Emery and Brown, 1958
Hierochloë alpina	Weimarck, 1970
" *australis*	Weimarck, 1967a
" *monticola*	Weimarck, 1967b
" *odorata*	Norstog, 1963; Weimarck, 1967a
Panicum maximum	Warmke, 1954
	Snyder, 1955; Bashaw and Holt, 1958;
	Reusch, 1961; Singh, 1965; Burson and
	Bennett, 1970, 1971; Burson, 1975
Pennisetum ciliare	Fisher *et al.*, 1954; Snyder *et al.*, 1955
" *orientale*	Chatterji, 1969
Poa ampla	Nygren, 1951
" *arctica*	Engelbert, 1940, 1941
" *arida*	Nygren, 1954
" *granitica*	Skalinska, 1959
" *pratensis*	Nielsen, 1946; Nygren, 1950
" *scabrella*	Nygren, 1954
Setaria leucopila	Emery, 1957
" *villosissima*	Emery, 1957
Sorghum bicolor	Hanna *et al.*, 1970
" *vulgare*	Rao and Narayana, 1968
Themeda triandra	Brown and Emery, 1957

TABLE 2 (Continued)

II. SPECIES WITH GONIAL APOSPORY
(Amphimixis may also occur)

Eragrostis curvula	Voigt, 1972
Poa alpina	Müntzing, 1933; Håkansson, 1943; Nielson, 1946
" *glauca*	Gustafsson, 1946-7; Nygren, 1954
" *nemoralis*	Müntzing, 1933
" *palustris*	Nygren, 1954
Saccharum officinarum	Nygren, 1954
" *spontaneum*	Nygren, 1954
Tripsacum dactyloides	Farquharson, 1955

B. NON-PSEUDOGAMOUS

I. SPECIES WITH GONIAL APOSPORY

Calamagrostis chalybaea	Nygren, 1946, 1954
" *lapponica*	Nygren, 1946, 1954
" *purpurea*	Nygren, 1946, 1954
Nardus stricta	Rychlewski, 1961
Poa nervosa	Grun, 1955

II. SPECIES WITH SOMATIC APOSPORY

Cortaderia jubata	This thesis
" *rudiuscula*	Preliminary observation[1]

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[1] See Addendum to Part 1 of this thesis.

functional pollen is known from one region and here sexual reproduction is possible. The position in *Nardus stricta* is similar.

Pseudogamy usually involves fertilization of the central nucleus (Rutishauser, 1967), but this has been conclusively established in grasses rather infrequently. Hair (1956) demonstrated fertilized endosperm in *Agropyron* (by diploid pollen giving hexaploid endosperm), and Hakansson (1943) in *Poa alpina* (by haploid pollen giving pentaploid endosperm). Reddy and d'Cruz (1969) demonstrated the independent union of one or both of the polar nuclei with male gametes; the endosperm, therefore, being triploid and the product of one or both polars.

The development of endosperm from unfertilized polars has been rarely reported; it is inevitable in *Cortaderia jubata* and also in *Poa nervosa*, and is general in *Tripsacum triandra* (Farquharson, 1955) in which species triple fusion is said to occur infrequently, if ever. Similarly, in *Nardus stricta* (Rychlewski, 1961) the endosperm usually develops from fused polar nuclei, but pollination is possible and several combinations of male gametes and polar nuclei have been observed. In *Poa nervosa*, as in *Cortaderia jubata*, the polars normally fuse before endosperm formation begins.

In pseudogamous grasses the segmentation of the egg cell is often autonomous and can be seen to precede anthesis or the arrival of the pollen tube (e.g., *Paspalum secans* Snyder, 1955; *Poa pratensis*, Tinney, 1940; Nielsen, 1947; *P. alpina*, Håkansson, 1943; *Agropyron scabrum*, Hair, 1956; *Tripsacum dactyloides*, Farquharson, 1955). Nevertheless, in all these species pollination is required for full

development of the embryo, since without it, endosperm fails. Division of the egg cell is reported as subsequent to, or simultaneous with, early endosperm formation in *Panicum maximum* (Warmke, 1954), *Pennisetum ciliare* (Snyder *et al.*, 1955) and *Themeda triandra* (Brown and Emery, 1957).

It is also clear from Table 2 that in the greater number of grass apomicts, the embryo sac arises from somatic cells of the nucellus. In fewer grasses the embryo sac may arise directly from the megaspore mother cell or from some product of imperfect meiosis. Besides those grasses with gonial apospory listed in Table 2, Brown and Emery (1958) suspect this condition occurs in *Fingerhouthia africana* and three species of *Eragrostis*.

Species with highly versatile reproductive mechanisms such as *Poa pratensis* and *Bothriochloa intermedia* have proved of particular interest in discussions on the role of apomixis in the evolution of species. Understanding of this aspect of apomixis has undergone some interesting developments. At first, apomixis was considered an evolutionary blind-alley, which might confer short-term benefits to a biotype particularly suited to current environments. This restricted viewpoint has given way to the recognition of a more complex and constructive role for apomixis in which it acts as an addendum to, rather than as a replacement for sexuality. Extensive breeding programmes in groups of grasses with sexual and apomictic species and biotypes (Clausen and Hiesey, 1958; Müntzing, 1940; Nygren, 1946). Harlan *et al.* (1964) have revealed complexes in which hybridity, polyploidy and a balance between sexual reproduction and apomixis produce a very rich variation.

One of the most extensive of these genetical studies embraced species attributed to three genera, *Bothriochloa*, *Capillipedium* and *Dichanthium* (de Wet and Harlan, 1970a; de Wet and Stalker, 1975). These three genera are united by the bridging "compilospecies" *B. intermedia*. They include sexually reproducing diploids and apomictic polyploids, and within *Bothriochloa* there are also sexually reproducing polyploid species. The net result of this very flexible combination of sexual and asexual reproduction at many levels of ploidy is a dynamic genetic system capable of sustaining a high degree of adaptive polymorphism and progressive evolution. Some of the difficulties of reconciling such fluid situations with the demands of formal taxonomy are discussed by Löve (1960), Davis and Heywood (1963), de Wet and Harlan (1970b) and de Wet and Stalker (1975).

The insight provided by these genetical studies has occasioned a reassessment of the role of apomixis. The most complete statements of the peculiar evolutionary properties of groups in which amphimixis and apomixis have persisted side by side are those of Clausen (1954), Heslop-Harrison (1961), Rutishauser (1967), de Wet and Harlan (1970a) and de Wet and Stalker (1975). These authors agree that the combination of sexuality and apomixis has allowed the formation of a greater number of distinct forms in nature than could the sexual process alone, and that these genetically distinct forms are fitted to the available ecological niches. This great ecological success points to the flexibility of the genetic system possessed by amphimictic apomictic groups. Isolation between sexual

species may be maintained with gene-exchange possible at higher levels of polyploidy, so that successful gene combinations may be propagated rapidly while the advantages of seminifery are retained. The rigidity of an apomictic breeding system is ameliorated by the retention of sporadic sexuality. Unreduced embryo sacs, or reduced sacs of polyploids, may develop either sexually or asexually and the balanace between these alternatives may be under seasonal or other environmental control. A changing environment, therefore, may directly induce the variability required for survival. Evidence of shifts in the balance between sexuality and apomixis under environmental influences are given by Nygren (1946), Grazi *et al.* (1961), Hjelmqvist and Grazi (1964), Knox (1967) and Saran and de Wet (1970).

The possibility of applying apomixis in the breeding of fodder grasses and cereals has stimulated much research (Fryxell, 1957). In recent years in both Russia and the United States considerable effort has gone into the development of apomictic strains of maize by means of crossing with *Tripsacum* (de Wet and Harlan, 1973).

MATERIALS AND METHODS

PLANT MATERIAL

Panicles of the apomictic *Cortaderia jubata* were gathered from plants growing at Botany Division, D.S.I.R., Lincoln (Fig. 1). They were raised in 1971 from seed from two sources: one, from plants naturalized in California and collected by Dr D.W. Cooper of the University at Eureka (Connor, 1971), the other from plants naturalized along the Waitakere Scenic Drive, Auckland.

The sexual species used in this study for comparative purposes were New Zealand species *C. fulvida*, *C. richardii*, *C. splendens* and *C. toetoe*, and the South American *C. araucana* and *C. selloana* and an F_3 hybrid between the two. All were authenticated plants in the collection of Mr H.E. Connor, Botany Division.

FIXATION

Preliminary work on the process of apomixis was first carried out using the classical technique of fixation in formo-acetic alcohol. The disappointing technical quality of the results, combined with the complexity of the structures to be elucidated, eventually led me to discard this method as inadequate.

Fixatives used in an attempt to obtain material adequately fixed for electron microscope study are listed below. Details of the results obtained with *Cortaderia* are

discussed later.

1. Standard aqueous potassium permanganate: 2% solution (Mollenhauer, 1959).
2. Standard Glutaraldehyde (Biological Grade): 3% and 6% in Sorensen's phosphate buffer. Postfixed in 2% osmium tetroxide.
3. Osmium tetroxide: 2% aqueous or 2% in phosphate buffer as fixative following glutaraldehyde or combined with potassium dichromate in Dalton's solution (Dalton, 1955).
4. Glutaraldehyde and Paraformaldehyde (Karnovsky, 1965). Postfixed in 2% osmium tetroxide (as modifed in Glauert, 1975).
5. Acrolein: 10% in 0.025 M phosphate buffer. Postfixed in 2% osmium tetroxide (Glauert, 1975).
6. Acrolein - Glutaraldehyde: 1% acrolein and 2.5% glutaraldehyde in 0.025 or 0.1 M buffer. Postfixation in 2% osmium tetroxide (Glauert, 1975).
7. Acrolein, Paraformaldehyde and Glutaraldehyde: 10% acrolein, 6% paraformaldehyde, 10% glutaraldehyde in 0.1 M phosphate buffer. Followed by postfixation in 2% osmium tetroxide (Hayat, 1970).
8. Osmium tetroxide - Potassium dichromate: 1% osmium tetroxide and 0.85% sodium chloride (Dalton, 1955).

Fixations using formo-acetic alcohol and later potassium permanganate and glutaraldehyde were carried out under reduced pressure (provided by a water vacuum pump). Later, the use of more powerful and pungent chemicals, some highly volatile, made this step in technique impracticable.

DEHYDRATION

After formo-acetic alcohol fixation, dehydration was carried out in a graded series of tertiary-butyl-alcohol.

Electron microscope fixation methods were followed by dehydration in a graded acetone or ethyl alcohol series. During the course of the investigation a complete series of solutions from 10% to 100% concentrations of the dehydrating agents by 10% steps were employed, to make the process more gradual, but these were later found to be unnecessary. Steps using 25%, 50%, 75%, 95% and 100% were found adequate, with the use of molecular filters in the anhydrous solutions.

EMBEDDING

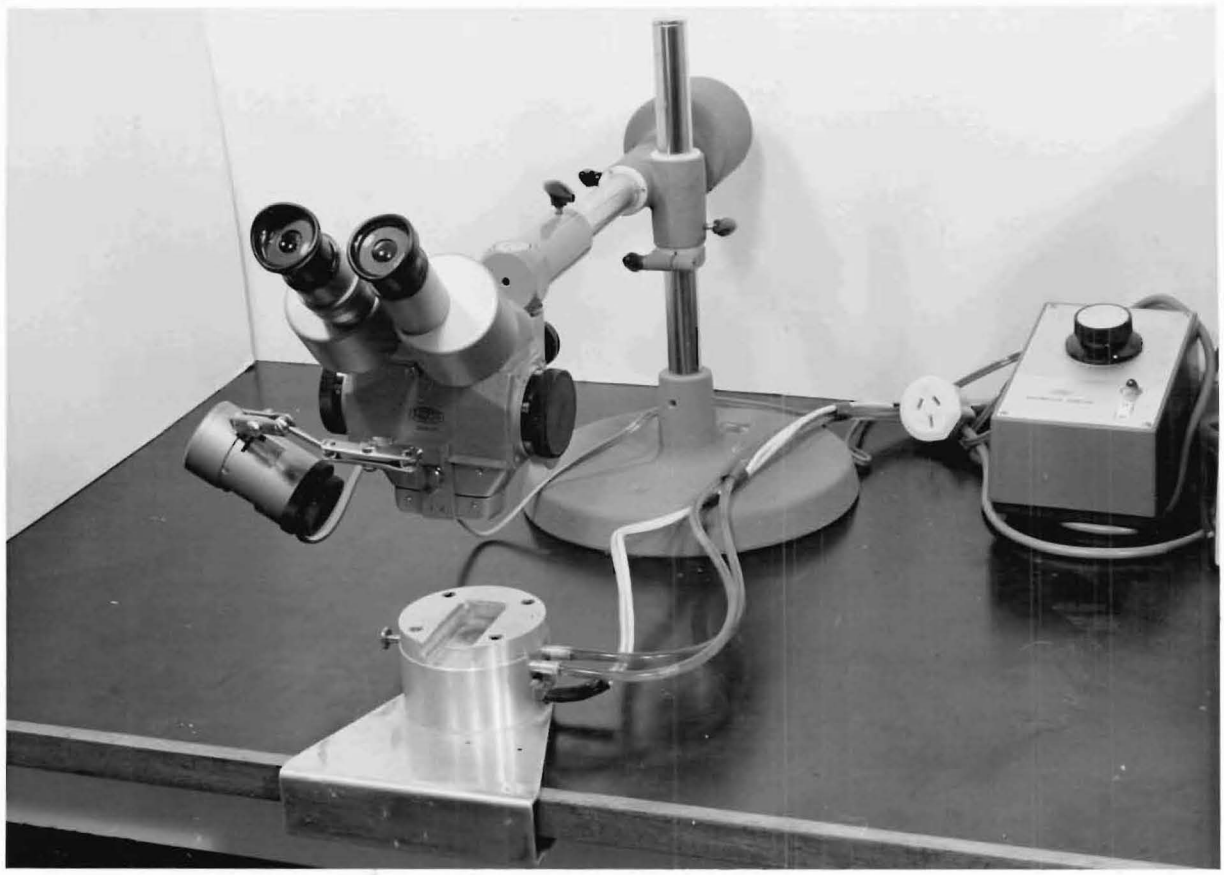
Formo-acetic alcohol fixed material was embedded in paraffin wax ('Paramat') after orientation in the instrument designed for the purpose and depicted in Fig. 2. This instrument was developed after finding that orientation of the ovary was necessary for sagittal sectioning of the ovule which lies in a vertical plane between the two style arms. Re-orientation of resin-embedded specimens had to be made after these had been embedded in BEEM or gelatine capsules. Later the acquisition of a microtome chuck designed to hold flat-embedded specimens eliminated a processing step and saved time in the preparation of material for the microtome.

Material for resin embedding was taken into propylene oxide and then into increasing concentrations of the resin before being orientated under a stereo microscope and then polymerized in the oven. The embedding media used were

Fig. 2

Apparatus for orientation of specimens in wax. A stainless steel dish (Tissue-Tek design) containing the specimen to be embedded is filled with molten wax and gently pushed into the rectangular space in the block. Here the bottom of the dish is in contact with a hot spot operated from a foot switch. Correct orientation is made under the stereo microscope using heated needles, while the wax is kept molten by momentary depressions of the switch. Rapid cooling of the wax is effected by pulling out the knob on the left, thereby sliding the hot spot from under the embedding dish and bringing the dish into contact with a water cooled area of the sliding panel. Solidification of the wax is rapid and with the specimen held firmly in the required position. The wax may be re-melted, if desired, and the operation repeated, by returning the hot spot to the central position under the embedding dish and depressing the foot switch.

This apparatus was designed and constructed by Mr I.A. Johnson, Botany Department, University of Canterbury.



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Araldite 502 (Fineran and Bullock, 1972), Epon 812 (Luft, 1961) and methacrylate-styrene (Mohr and Cocking, 1968). Material embedded in Araldite and Epon were contained in flat embedding dishes; those in methacrylate, in gelatin capsules filled to the top and capped. Polymerization was carried out at the temperatures and for the times recommended for the various mixtures.

MICROTOMY

Specimens embedded in Paramat were sectioned on a Spencer Rotary Microtome.

Those in epoxy resins or methacrylate-styrene resin were either cut on an LKB Pyramitome at 3 µm in serial section, or on an LKB Ultratome II for thin sections using glass or diamond knives.

Some sections were cut at 4 µm on the Pyramitome, examined, photographed and re-mounted on the prepared ends of BEEM capsules and re-sectioned for electron microscope examination, following the technique of Woodcock and Bell (1967).

STAINING

Paraffin sections were stained in Safranin and Fast Green or in Sharman's stain (Sharman, 1943), and mounted and covered in the usual way.

Sections for thick-thin sectioning and for ultramicrotomy were block stained overnight during dehydration in saturated uranyl acetate in 70% acetone, or in 1% uranyl nitrate in 70% acetone. Later, sections were post stained

with lead citrate (Reynolds, 1963), either on Sjöstrand Type one-hole grids or on 100 mesh copper grids. Grids were floated on drops of lead citrate contained in a petri dish on a clean piece of Parafilm, and a few pellets of sodium hydroxide were added to the petri dish to prevent carbon dioxide contamination. Ultrathin sections of *Cortaderia* were found generally to give poor contrast under the electron microscope. Staining with uranyl acetate was often done after sectioning, followed by post staining with lead citrate, with no improvement. Methylated uranyl acetate was also tried, but it was found each time that sections floated off the grid. The method was therefore abandoned.

Best contrast was obtained with the use of 2% potassium permanganate (Fineran and Lee, 1975). While contamination was a disadvantage with this method, the increased contrast in micrographs outweighed this.

Sections cut at 3 µm were stained with Methylene Blue - Azure Blue combination (1:1), 1% aqueous Safranin, or Paragon Polychrome Stain. These sections were then mounted in Depex or Permount and covered.

GRIDS

Ultrathin sections were picked up on Sjöstrand Type one-hole grids, stained and then mounted on 100 mesh copper grids, or picked up directly on mesh grids. Grids were covered with a colloidin film reinforced with carbon, or Formvar coated.

MICROSCOPES

Optical studies were carried out on Leitz Ortholux and Reichert Zetopan microscopes.

An Hitachi HS-7 electron microscope was used for all ultrastructural observations.

Dissections were carried out with a Circon micro-dissection scalpel and needle under a Wild stereomicroscope M 5.

PHOTOGRAPHIC PROCEDURES

Electron micrographs were made on Agfa Scientia photographic plates, developed in Microphen and fixed in Amfix. Prints were made on Agfa papers, using an Agfa Rapidoprint.

DIFFICULTIES ENCOUNTERED IN PROCESSING, AND THEIR INVESTIGATION

Young spikelets, or larger single ovaries, fixed in formo-acetic-alcohol and embedded in paraffin, yielded specimens which were of poor quality, showing a great deal of shrinkage and distortion of the embryo sacs and their contents. This method, however, yielded information which formed the basis of an understanding of the apomictic process in *C. jubata.*

Investigations were continued into the problem of fixation of material for ultrastructural studies and for the improved quality which glutaraldehyde and other electron microscope fixatives are claimed to show over F.A.A. Initially, ovaries from freshly gathered panicles at all

stages of development were dissected out from the palea and lemma, and fixed in glutaraldehyde or glutaraldehyde - paraformaldehyde (Karnovsky's solution), postfixed in osmium tetroxide, dehydrated in a graded acetone or alcohol series and embedded in Araldite. It was found, however, that practically all but the smallest specimens (and sometimes even these) showed varying degrees of bad fixation, such as complete collapse and browning of internal tissues of both ovary and ovule, the random occurrence of large or small areas of such denatured tissue through the specimen, and commonly, the reduction of the entire ovule centre to an unimpregnated hollow core.

A list of the solutions tested is given under Fixation earlier in this section, and Table 3 summarizes the first series of investigations, in which standard techniques were used.

Variations were made in the methods, as indicated in Table 3. In addition, a check was made to ensure that careful and complete dehydration (using molecular filters in the final steps) was obtained. A very slow infiltration of Araldite was made with a number of transitional steps in varying proportions of propylene oxide: resin, and small amounts of resin were added, freshly prepared for each step in infiltration, so that the process lasted approximately one week.

There was no appreciable increase in the number of ovaries successfully fixed. Hundreds of specimens of *C. jubata* and the New Zealand sexual species *C. fulvida*, *C. richardii*, *C. splendens* and *C. toetoe* and the South American *C. araucana* and *C. selloana*, many in both hermaphrodite and

TABLE 3

Details of Initial Fixation Tests

Specimen	Fixative	Time	Under vacuum	Post fixative	Embedding medium	Results
Whole ovary	GA, 3% & 6%	5 - 24 hrs	½ - 5 hrs	OsO_4 2% 2 - 24 hrs	Araldite	Ovules with hollow centres
" "	GA + Form. (Karnovsky)	24 hrs	2 hrs	OsO_4 2% 24 hrs	"	" " " "
" "	$KMnO_4$ 2% aq.	2 - 24 hrs	1 hr	--	"	" " " "
Style arms removed	GA, 6% GA + Form. (Karnovsky)	24 hrs	"	OsO_4 2% 24 hrs	"	" " " "
Ovule (dissected from ovary)	GA, 6%	"	"	"	"	Some ovules fixed but orientation a problem

GA = Glutaraldehyde

Form. = Paraformaldehyde

female forms, all showed similar resistance to fixation.

The complicated leaf waxes of *Cortaderia* have been the subject of research (Martin-Smith *et al.*, 1967, 1971) and it was felt that fixation difficulties possibly could be due to a heavy coating of waxes on the ovary. In an attempt to remove this, ovaries were treated by dipping them in chloroform for periods from 2-10 seconds, before plunging them into fixative. Although they all appeared more opaque after this treatment, no appreciable improvement in the number successfully fixed was evident.

There was a possibility that ovules with hollow centres were the result of the embedding medium failing to penetrate the tissues. Tests were therefore made using media of low viscosity, as listed in Table 4.

Spurr's resin was investigated as it is an epoxy resin of low viscosity. However, this medium failed to polymerize correctly on two separate occasions, and many specimens were lost. The method was abandoned.

Epon, prepared according to the method of Luft (1961) was also tried, but penetration of the specimens was no better than with Araldite.

An inconvenience of the methacrylate-styrene medium is that polymerization must be carried out in filled and closed capsules to exclude air which inhibits the process (a number of attempts to use other, flat containers were unsuccessful) so that specimens required re-orientation after polymerization. It was found, however, that quite a high proportion of ovaries were successfully fixed; the medium cut well and contrast under the electron microscope was good.

TABLE 4

Tests to Investigate Efficacy of Infiltration Using Resins of Low Viscosity

Specimen	Fixative	Fixation time	Evacuation time	Post fixative	Embedding medium	Results
Ovaries larger than 0.5 mm, decapitated	2% GA	3 hrs	½ hr	OsO_4 2%, 3 hrs	Methacrylate - styrene *	Good fixation of a number of ovaries (later found to be mainly due to plant used being more amenable to fixation
"	6% GA	24 hrs	1 hr	OsO_4 2%, 24 hrs	Epon (Luft, 1961)	Ovaries with hollow centres
"	6% GA	"	"	"	Spurr's Medium	Medium polymerized to brittle consistency which was impossible to section

* A series of different mixtures of n-butyl and methyl methacrylates was prepared to determine the requisite degree of hardness. This was found to be in the ratio of 4 : 3.

The method of embedding in methacrylate-styrene appeared at the time to be the answer to the processing problem posed by these ovaries and ovules. Consistently poor results were masked by the fact that, unaccountably, occasional specimens would show good fixation. It came to be realised during the course of this study, that plants of this genus can differ in their resistance to fixation. In regard to *C. jubata*, there are twelve plants in the plot and all appear morphologically identical. However, while most of these are extremely difficult to fix, one or two are slightly more amenable. Material from one of these plants had unsuspectingly been used in the test with methacrylate-styrene. Fixations using other plants were not successful.

Efficacy of fixation is difficult to estimate, as this can be judged only after an ovule has been sectioned. With resin-embedded specimens this is a slow and laborious task, involving the handling of many separate 2-3 µm sections, as ribboning does not occur.

A return was made to the testing of a range of fixatives in the hope that a method of chemical penetration could be found, without having to resort to dissection of such small specimens with its attendant difficulties.

At this point in the account of the technical processes used in this investigation, it may be of interest to record the methods by which very small specimens were handled. Developmental studies required ovaries to be processed, ranging from approximately 0.1 mm to 1.5 mm. Each time many were lost during processing, and in the earlier steps most ovaries floated (despite fixation under

reduced pressure and removal of the style arms) making withdrawal of solutions difficult. Attempts to minimize losses were made in the following ways:

(a) BEEM capsules were modified to small strainers, with an electron microscope grid welded into the shaped top after the method of Buchanan (1965). Three capsules were stood firmly together in a 5 ml beaker and fixatives and dehydrating solutions added to or withdrawn from the beaker. This kept specimens confined from liquids being withdrawn, so they could not be drawn into the Pasteur pipette and adhere to the inside. However, the method did nothing to help them to become immersed.

(b) An improvement was made by welding an electron microscope grid into the BEEM capsule lid and fitting this after the specimens had been placed inside, these actions being carried out while the capsule was standing in fixative in a small beaker. Once the capsule lid was fitted it was necessary to hold the capsule in forceps upright and just under the surface of the processing liquid until it was seen that the liquid had risen to the top and only a very small air bubble was present.

Methods (a) and (b) proved satisfactory for passing small specimens through a series of processes. Losses were minimized, but the method did require specimens to be removed into another container before infiltration began. Also, to fill a sealed BEEM capsule required much more than the usual amount of osmium tetroxide, in fact 2 ml per

capsule were required to fill this to the top and then capsules had to be kept immersed. In the interests of economy these methods were abandoned in favour of that involving dissection of the ovary and wounding of the ovule to allow penetration of the chemicals. The method is described a little later in this section.

Table 5 summarizes the fixatives used in the second series of tests and the results obtained. The embedding medium was changed from Araldite to Epon because of the latter's lower viscosity and apparently consistent penetration of tissues whenever fixation was adequate. The use of methacrylate-styrene was discontinued because of the care required in its preparation, the pungency and unpleasant nature of its constituents, and the need for re-orientation of specimens after polymerization.

As in the tests using methacrylate-styrene, one apparently favourable result later proved to be due to the specific nature of the plant material used. An F_3 hybrid *(C. araucana* x *C. selloana)* was later found to give a relatively high proportion of adequate fixations with the more powerful fixatives, often without any wounding having been carried out on the ovary. This did not hold for *C. jubata.* Unfortunately, the exploratory nature of this work, the time involved in obtaining results, and the shortness of the annual flowering season made it impossible to carry out all tests using the same species of *Cortaderia.* Results obtained from tests on other species of *Cortaderia,* which have different flowering periods, were used in an attempt to fix ovaries of *C. jubata,* often in the following flowering season.

Removal of the ovule from the ovary was an obvious

TABLE 5

Testing of a Second Range of Fixatives

Specimen	Fixative	Fixation time	Post fixative	Embedding medium	Results
Whole ovary	Acrolein 10%	24 hrs	OsO_4 2% 24 hrs	Epon	Good fixation of hybrid *Cortaderia*, otherwise poor or sporadic
"	Acrolein 1% + Glutaraldehyde 2.5%	"	"	"	Sporadic, mainly low proportion
"	Acrolein 10% Paraformaldehyde 6% Glutaraldehyde 10%	"	"	"	" " " "
"	OsO_4 1% K_2CrO_7 1% NaCl 0.85%	"	"	"	" " "

method of exposing it to fixatives. After many hours' work a number of "large" ovules were removed from ovaries, with varying degrees of dissection damage. Some of these ovules were unavoidably lost during subsequent processing as they were practically invisible to the naked eye. Also, it was found that osmication greatly reduced the chances of correctly orientating the ovule under the stereo microscope, as the specimens took on a uniformly black colouration so that features of "side" and "front" were lost. Complete removal of the ovule from the ovary was a time-consuming task, but did increase the chances of better fixation. It was noticed that ovules showing dissection damage were often well fixed while others that were more or less whole usually showed the typical, badly fixed regions.

These results indicated that wounding of the ovule as well as of the ovary was necessary for fixative penetration to occur, and the following method was devised for the wounding of these small objects and their subsequent handling.

The main difficulty involved in dissection was that of holding still a very small, slippery and delicate object while attempting to slice the ovary without damaging the ovule in the region under study. This problem was more or less overcome by gently applying ovaries dissected from their glumes to double-sided Sellotape. Specimens were removed from rachillas under glutaraldehyde and collected in a holding drop on the dissecting plate. From there they were transferred, one by one, to a small strip of Sellotape near the holding drop. Ovaries were held in fine-pointed forceps by their style arms, placed on the doubled-sided

Sellotape and finally gently pushed down by means of a toothpick which had been made blunt at one end. The shape of the ovary made it impossible for it to be held firmly by the tape but the presence of the style arms greatly assisted in holding it down. This step of sticking the specimen on to the Sellotape had to be accomplished quickly, and then a drop of glutaraldehyde from a Pasteur pipette was used to cover the specimen. Each ovary was so attached to the Sellotape and covered with a separate drop of glutaraldehyde. It was found necessary to keep the drops of glutaraldehyde intact and not to allow them to drain off on to the dissecting plate or the ovaries would suffer drying out. Also, liquid lifted the Sellotape from the dish and made it an insecure base on which to dissect.

When the ovaries had all been applied to Sellotape and each covered with a drop of glutaraldehyde, which also acted as a lens and magnified the object, cuts were made in the ovary with micro-dissection instruments, the most satisfactory of these being a Circon micro-knife and micro-needle. Wounds were made in the ovary wall, usually along one side and the dissection tool was used to lift the tissue gently to ensure entry of the fixative. As the ovule lies in a plane vertical to that in which the ovary falls when removed from its glumes, this wounding caused the least amount of damage to ovules in the embryo sac region. A deep cut was made at the base of the styles to penetrate the ovule at that point.

When all ovaries had been so wounded, the strip of Sellotape was flooded with fixative and carefully raised from the dissecting dish (this was greatly facilitated by

fixative flowing between the strip and the dish) and the strip was placed in a vial of fixative for the requisite fixing period. All subsequent processes were carried out with the ovaries firmly attached to the Sellotape, and with all the hundreds of specimens processed in this way very few became detached from the tape.

At first the specimens were embedded still adhering to the Sellotape, which became diaphanous during processing and presented no difficulties in microtoming. However, it was inclined to float up during polymerization, which resulted in ovaries becoming disorientated. Also, too many ovaries adhering to a strip made later cutting out of the blocks difficult. Consequently, specimens were carefully removed from the tape at or immediately after the 70% dehydration stage (stigmata were often lost during this operation), and were then returned to their container where they sank to the bottom and could be easily observed (now being black after osmication) during withdrawal of the solutions.

Specimens were finally embedded in flat plastic lids.

Wounding of ovaries and ovules, combined with the use of the more powerful fixatives listed in Table 5, has been the method by which material has been prepared for this study. Fixation, however, has remained sporadic and unpredictable, with differences between individual plants of *C. jubata* showing in the proportions of ovules successfully fixed. One plant out of twelve gave the best proportion of fixation of ovules - 30% is perhaps a generous estimate. All the New Zealand species were extremely difficult to fix, as was the South American *C. araucana*. *C. selloana* was a

little more amenable and the hybrid between the two South American species proved much easier on preliminary tests. However, it can be said that when fixation occurs the quality of preservation is reasonably good. Acrolein seems to increase penetration with good preservation of cell components. If penetration occurs, glutaraldehyde alone or with paraformaldehyde gives adequate fixation and is easier to work with than the pungent Acrolein. Dalton's fixative using potassium dichromate was tried at the end of the study with very little success, and observations were hardly sufficient to pronounce on the efficacy of fixation by this method.

The sporadic nature of fixation has made it impossible to gauge the quality of preservation of the various fixatives from the ultrastructural aspect. Differences in quality undoubtedly exist, as is evident from the electron micrographs, but such differences, of course, could as readily be a function of individual cells, especially embryo sac components, as of the action of a particular fixing agent. Sporadic fixation was also responsible for the failure of attempts to determine the effects of differences in buffer osmolarity.

PART I

THE MECHANISM OF APOMIXIS

1. THE OVULE

The ovule of *Cortaderia jubata* is hemianatropous with the micropyle inclined towards the base of the loculus, bitegmic and tenuinucellate (Fig. 12). In initial stages of development the ovule is inserted on a broad placenta on the ventral side of the ovary and is virtually erect (Fig. 3), but as development proceeds the placenta occupies a decreasing proportion of the wall and the curvature of the ovule increases (Figs 10-12). These changes in form are largely due to growth of the chalazal tissue, especially on the side towards the styles.

The Integuments

The inner integument originates as a meristem surrounding the base of the very young ovule primordium at a time when the megaspore mother cell is not yet fully differentiated (Fig. 3). This meristem originates by enlargement of and divisions in protoderm cells. The outer integument arises just after the archesporial cell becomes clearly defined (Fig. 4). Divisions occur in the surface and sub-surface cells at the base of the outer face of the inner integument. Both integuments remain two cells thick, except for their basal portions and the bulbous ends of the inner integument which begin to thicken as soon as the micropyle is defined. The inner integument always remains

longer than the outer but does not completely enclose the nucellus until the somatic embryo sacs are two-nucleate (Fig. 10). In the maturing ovule a vertical process develops from the outer integument which penetrates up the canal between the two style arms.

The Nucellus

In its early stages the nucellus is more or less hemispherical but broader than high (Figs 3-4). By increase in cell number and especially by an increase in cell height (including the megaspore mother cell), the nucellus soon becomes cylindrical (Figs 5-9). By the time cell division begins in the embryo sac, downward curvature has begun and is continued mainly by proliferation of the chalazal region, emphasizing the hemianatropous form of the ovule (Figs 10-12). The enlargement of some nucellar cells as embryo sacs and the degeneration of other nucellar cells will be described later. The archesporial cell transforms directly into the megaspore mother cell (without the production of parietal cells) and the protoderm at the apex of the nucellus also remains without periclinal divisions. The ovule is, therefore, tenuinucellate.

2. THE EMBRYO SAC

The megaspore mother cell and the failure of meiosis

An archesporial cell can be distinguished in the sub-epidermal layer of the nucellus at a stage before the initiation of the outer integument (Fig. 3). This becomes a fully developed megaspore mother cell before the micropyle is fully closed (Fig. 5). It is an elongated cell, broader

at its distal end where the large nucleus is situated, and with walls that colour deeply in Sharman's stain. Degeneration of this cell may begin before the nucleus enters prophase of the first meiotic division, or after more or less clear preliminaries of this division may be observed. Only one instance of meiosis proceeding further was encountered. In that ovule, degeneration material lay above a cell with an elongated nucleus. It was not possible to determine whether this represented a dyad or a tetrad (Fig. 6). Degeneration of the whole cell or its meiotic products rapidly sets in (Figs 7-9). This process is associated with the appearance of darkly staining bodies below the micropyle in the degenerating cell and its neighbours. The collapsed cell or dark fragments of its disintegration can be detected below the nucellar cap during much of the subsequent development of the ovule.

Origin and organization of somatic embryo sacs

As megaspore mother cell degeneration begins, a varying number of neighbouring nucellar cells enlarge, usually becoming elongated (Figs 7, 9). Initially these are located near the micropyle, though subsequently they often arise nearer the chalazal region. Five or six nucellar cells may enlarge and begin development as embryo sacs, but it is usual for only two or three to reach later stages of development (Fig. 12). Occasional ovules are found in which no embryo sac has developed. Estimation of the number of embryo sacs present is often difficult when more than two occur, as these appear to be intimately associated with one another and intertwined so that their

relative positions change in successive sections. These difficulties are often aggravated by fixation distortions. Especially in F.A.A.-fixed material the outline of the embryo sac is often highly convoluted and invaginated. That this is a result of processing is indicated by the appearance of fresh ovules viewed by transmitted light. Under these conditions the embryo sacs appear as regular, bubble-like, clear spaces.

In most ovules two or three advanced embryo sacs will form, but normally only one will develop an embryo. Nevertheless, ovules with two embryos are not unusual (Figs 39-41), and a germination test showed that about 6% of seeds produced twin seedlings. As will be described later, embryo sacs which do not develop an embryo may continue to develop and play a significant part in the provision of endosperm in the seed.

The enlarging nucellar cells at first contain a single nucleus but this soon undergoes division. The two daughter nuclei come to lie at each end of the enlarging cell and are separated by a large vacuole (Fig. 10). Each of these nuclei then undergoes further division (Fig. 11) to form a rather variable number of nuclei which become organized into positions which permit them to be related with more or less precision to the nuclei of the 'normal' sexual embryo sac (Fig. 12). Embryo sacs with from three to eight nuclei have been observed in *C. jubata*, but six may be considered normal — two antipodals, two polar nuclei, one synergid and one egg.

The polar nuclei

Two cells above the centre of the embryo sac and lying one above the other on its long axis are equivalent to the polar nuclei of normal megagametophytes (Figs 12, 19). In some cases there is evidently only one polar nucleus, and in a few none. The latter, at least, would be unable to develop endosperm. By the time the sac is mature the two polar nuclei may have fused to form a single, large, central cell nucleus which has a very evident and large nucleolus (Figs 13, 14). Central cell nuclei with up to five nucleoli have been observed. While in F.A.A.-fixed material the central cell can be well separated from the egg apparatus, in glutaraldehyde-fixed material it is always closely associated with it, and this is considered the more natural location.

The original embryo sac wall becomes the wall bounding the central cell, except where it abuts on the antipodals and the egg apparatus. This wall is characterized by a feature which enables it to be readily identified from other walls within the ovule. Its inner surface is covered with peg-like projections, often quite long and just visible under the light microscope (Fig. 15), which are more richly developed in some areas than in others. These projections (transfer walls) will be fully discussed in the section on electron microscopy.

The antipodal cells

The antipodal cells usually form a compact group of four or more cells, frequently lying against one lateral wall of the embryo sac and arranged in the form of a

crescent (Fig. 15). Frequently, they can be seen to form two groups, presumably derived from the original two cells. Occasionally they occupy a position at the chalazal end, and in several ovules they have been located at the micropylar end (Fig. 16), in which case the organization of the whole embryo sac is inverted (Fig. 24). When multiple embryo sacs occur, the antipodals of the one lower in the ovule may lie adjacent to those of the micropylar sac, so that polarity of the sac is again inverted. Less frequently, the antipodals form a sub-spherical mass (Fig. 17), usually in a caecum-like protrusion at the chalazal end of the embryo sac (as is usual in the sexual species described below). Intermediate states occur and rarely antipodals appear to be absent. The antipodal cells are formed early in the development of the embryo sac and some enlarging sacs may produce a group of antipodal-like cells and progress no further. Their function within the embryo sac must be of some importance not only because of their early prominence but also because of their retention during the early development of the embryo (Fig. 24). Transfer walls are a regular feature of the antipodals, but these are confined to the outer walls. When the antipodals are laterally located, they frequently give the appearance of lying outside the embryo sac. That is to say, the wall between these cells and the interior of the embryo sac is thicker than the outer wall. However, the presence of transfer processes on the outer walls of the antipodals indicates that they lie within the embryo sac.

Some ultrastructural aspects of the antipodal cells will be described in Part II.

The egg cell

The nucleus which is analogous to the egg cell has distinctive cytological features which will be discussed in greater detail in the section on ultrastructure (Part II). As the embryo sac matures, the egg nucleus becomes positioned between the central cell nucleus and the micropylar end of the sac, where it is always closely associated with the synergid (Figs 13, 14). The egg nucleus develops a cell wall and its cytoplasm becomes highly vacuolate and characteristically contains numerous dark bodies. The prominent centrally located nucleus appears stellate under the light microscope and this is confirmed under the electron microscope (see Part II). The conspicuous nucleolus is not homogeneous, but displays areas of lighter staining material. The cytoplasm of the egg occasionally extends directly into the haustorium (see below).

The synergid and haustorium

Associated with this egg cell is one analogous to a synergid (Figs 13, 14). In F.A.A.-fixed material it is usually separate from the egg cell, but in glutaraldehyde-fixed material the two cells are intimately associated and the wall between them often appears incomplete. The haustorium is a finger-like process which penetrates the micropyle before turning to one side between the outer integument and the ovary wall (Figs 13, 14, 29). The haustorium may extend in both directions from the micropyle, and may appear to branch, but when viewed under the electron microscope a more or less clearly separated second synergid is seen to be responsible for this appearance.

The haustorium develops at the time of organization of the embryo sac. A chalazal nucellar cell developing late in an ovule already with a mature embryo sac, bore an extension that could be regarded as an haustorium (Fig. 18). In a few ovules (fixed with both F.A.A. and glutaraldehyde) the cytoplasm of the egg also appears to have organic connection with the contents of the haustorium. Indeed, in a number of ovules, the egg itself develops an haustorium alongside that of the synergid (Fig. 19).

The haustorium is most active in the early stages of maturity when the finger-like projection appears, under the light microscope, to contain heavily granular cytoplasm and to bear obvious long transfer processes on its walls. Clearer, less granular regions are also visible, but these may be artifacts due to processing, or brought about by rapid extension of the haustorium, or by other physical or biochemical agencies. With advancing maturity, the haustorial cytoplasm becomes increasingly less homogeneous in appearance and with larger areas of translucence (Fig. 20), until eventually with the growth of the embryo beyond the globular stage, the haustorium can be detected under the light microscope only as a crushed remnant adjacent to the suspensor of the embryo (Fig. 33). Occasionally, especially in F.A.A.-fixed material, a ghost-like impression of a completely translucent synergid and haustorium devoid of contents may be seen adjacent to older embryos (Fig. 37). There is always well developed endosperm in these embryo sacs.

As observed under the light microscope, *C. jubata* normally has only one synergid, which is haustorial in the

great majority of cases. Studies under the electron microscope (Part II) disclose traces of a second synergid. Very occasionally two well formed synergids may be present, in which case both will normally bear an haustorium which penetrates the micropyle (Fig. 21).

The situation with multiple embryo sacs becomes quite complex. Where a developing embryo sac lies near the micropyle, it will produce an extension through the micropyle. Alternatively, an haustorium may reach the space between the integuments and the ovary wall by lateral penetration of the tissues of the nucellus and integuments (Figs. 22, 23). In one specimen, the synergid of an embryo sac immediately below the micropyle had developed an haustorium extending laterally because the entrance to the micropyle was blocked by an embryo of another embryo sac, already many-celled and globular (Fig. 24). No more than two well-developed haustoria in one ovule have been observed, even in ovules bearing a number of embryo sacs, and the possibility of sacs benefitting nutritionally from the activity of adjacent haustoria has often appeared to be a real one. In the ovule represented in Figure 25 two embryo sacs, both large and well-developed, lie closely appressed. One sac contains an undivided egg and has an haustorium through the micropyle. The other, while apparently without a synergid or haustorium of any kind, bears a young two-celled embryo. The position of attachment of this embryo to the embryo sac is well defined but there is a broad extension from it into the micropyle and contiguous to the haustorium but apparently separate from it. It would appear that, either the young embryo has the

capacity to develop an haustorium or, at least, is able to benefit from the proximity of the adjacent haustorium. The potential of the embryo to develop an haustorium is upheld by the strong impression that even more advanced embryos than this one may bear extensions of the basal cell which may reach quite considerable lengths and penetrate the ovular tissue.

3. EMBRYOGENESIS

Endosperm formation

Products of division of the central nucleus form a coenocytic layer around the periphery of the enlarging embryo sac (Fig. 26). Similar cells soon surround the egg cell (which may have begun to divide by this time). This free-nuclear stage of endosperm development soon passes into the cellular stage. The cells divide freely and invade the large, clear space within the enlarging seed (Fig. 27). The starchy endosperm cells of the mature seed may show up to three zones. These are the result of embryo sacs, in which embryos do not develop, nevertheless forming dense masses of endosperm (Fig. 28). No doubt the reserves in all these zones will be available to the developing embryo. As is typical of grass caryopses, an aleurone layer is present in *Cortaderia*.

The embryo

Division in the egg cell may be delayed until several divisions have taken place in the endosperm (Fig. 26), but it is more usual to find a pro-embryo in a sac in which the central nucleus is still undivided. Early segmentation

of the "zygote" has not the regularity of sexual species, nevertheless, young embryos may closely resemble proembryos (Figs 29-31). At an early stage, the cell nearest the micropyle with less dense cytoplasm resembles a suspensor cell (Fig. 29) and it may persist, playing no part in the construction of the embryo. This cell retains its original connection with the embryo sac wall, and also remains closely appressed to the remains of the haustorium. After the initial segmentation when a sub-spherical mass of cells has been formed (Figs 32-35), the development of the embryo follows the normal pattern (Figs 36-38). The final form of the mature caryopsis conforms to the pattern described for the Arundineae by Reeder (1957).

Two embryos, each developing from a separate sac, are not infrequent (Figs 39-41). These may be of similar size, or one may be markedly advanced and this is not necessarily the one closest to the micropyle. The two embryos may lie side by side below the micropyle or one may lie below and to one side of the other. Both embryos may mature successfully, and eventually germinate, or only one, which may lie in a lateral position, may mature.

4. COMPARISON WITH SEXUAL SPECIES

Before drawing a comparison between apomixis as found in *C. jubata* with that in other genera of grasses, it is necessary to know which features of the development are related to apomixis and which are characteristic of the genus. For example, is the synergid haustorium a feature of apomixis or of the genus? So far as is known, no study

of gametogenesis in *Cortaderia* has been undertaken previously. It was, therefore, essential to observe the principal features of the embryo sac of some sexual species. Those investigated were the South American *C. selloana*, its hybrid with *C. araucana*, and four New Zealand species, namely, *C. fulvida*, *C. splendens*, *C. richardii* and *C. toetoe*. These were studied only as regards the main features of the mature embryo sac. Since these were all essentially alike, a single generalized description will be sufficient. In addition, it was considered advisable to confirm the occurrence of normal meiosis, but this was done for one species only *(C. toetoe)*.

The ovule is tenuinucellate with the micropyle formed by the bulbous ends of the inner integument. Meiosis was not observed directly, but ovules of *C. toetoe* with apparent linear tetrads of spores (of which the three micropylar spores have degenerated) indicate that macrosporogenesis is normal in that sexual species (Fig. 42).

The mature embryo sac is spindle-shaped with a group of antipodals usually situated in a spherical caecum at the chalazal end (Fig. 43). The polars are typically fused before fertilization to form a large, central nucleus which usually lies closely associated with the two well-defined synergids. These are elongated, pear-shaped cells with the nucleus and highly vacuolated cytoplasm in the dilated end (away from the micropyle). Typically (but not invariably) the two synergids are prolonged as well-defined haustoria lying side by side, passing through the micropyle and projecting for a short distance between the integuments and the ovary wall (Fig. 44). These haustoria contain

densely staining granular cytoplasm and staining is particularly heavy at the apical ends, where wall structure appears to be massive. It is possible that the filiform apparatus is situated at the extended ends of the synergids. The nucleus of each synergid is no longer visible at maturity. The walls of the embryo sac and haustoria bear projections on their inner face, similar to those of *C. jubata*, which can be seen clearly under the light microscope. Irregular, lateral projections of the embryo sac into nucellar and integumentary tissue have been observed in a few ovules of *C. toetoe* but their significance is unknown.

It can now be seen that some of the features of *C. jubata* are shared by these sexual species. For example, they all develop synergid haustoria and all have prominent transfer processes on the inner face of the central cell and haustorial walls. A difference between *C. jubata* and the sexual species not directly related to apomixis is the location of the antipodals. In *C. jubata* these are mostly lateral while in the sexual species they are predominantly chalazal.

With this additional information it is now possible to make a more meaningful comparison with apomixis as reported in other grass genera.

5. DISCUSSION

The principal feature of the mechanism of apomixis in *Cortaderia jubata* is that the megaspore mother cell, or the products of imperfect meiosis, invariably degenerate.

The place of the normal embryo sac is taken by one or more unreduced embryo sacs which develop from nucellar cells. The mechanism of apomixis in the species is, therefore, somatic apospory, which is the condition present in the majority of grass apomicts (Table 2). Since no functional pollen is produced in *C. jubata*, apomixis is obligatory and seed-development is non-pseudogamous. All other non-pseudogamous grasses exhibit gonial apospory (see Table 2), therefore, *C. jubata* appears to be unique among apomictic grasses in the combination of its characters.

The sequence of divisions and the pattern of vacuolation in the nucellar embryo sacs of *C. jubata* are variable, as in many other apomictic grasses, nevertheless they clearly relate to the Hieracium-type pattern of development. That is to say, its megagametogenesis is of the Festucoid, not Panicoid type (Battaglia, 1963; Rutishauser, 1967). The Arundineae combine features characteristic of both subfamilies of grasses (de Wet, 1954; Reeder, 1957) but this particular character supports the view that the Tribe approaches most closely to the Pooideae (Avdulov, 1931).

Since most apomictic grasses are pseudogamous, fusion of a male gamete and the central nucleus (or polar nuclei) is known to occur, or is assumed (see introduction for references). Development of endosperm without such fusion has been demonstrated for few grasses. It is inevitable in *C. jubata* and *Calamagrostis chalybaea*, and also in *Poa nervosa*, *Nardus stricta* and *Calamagrostis purpurea* (except in regions where functional pollen is formed). Otherwise these fusions are reported as absent, or very rare, in *Tripsacum triandra*

(Farquharson, 1955). As in *Poa nervosa* (Grun, 1955), the polar nuclei of *Cortaderia jubata* normally fuse before endosperm formation begins.

The relative timing of the first divisions of the egg and of the central nucleus is variable in *C. jubata*. Even in the same ovule, in one embryo sac the egg may divide before endosperm formation sets in, while in a neighbouring sac the reverse may occur. Gustafsson postulated that apomixis might arise because precocious embryo formation precluded fertilization. This explanation could apply to most grass apomicts (including *C. jubata*). However, in several apomictic grasses the egg remains undivided until after the pollen tube reaches the embryo sac (*Panicum maximum*, Warmke, 1954; *Pennisetum ciliare*, Snyder *et al.*, 1955; *Themeda triandra*, Brown and Emery, 1957).

Tinney (1940) and Skalinska (1959) comment on the occurrence of small embryos in species of *Poa* before anthesis, but the formation of embryos in *C. jubata* appears to be much earlier than in any other grass. Divisions have taken place in both the embryo and the endosperm by the time the panicle is protruding from the upper leaf-sheaths, and by anthesis when the stigmas are spread, embryogenesis is well advanced.

Since *C. jubata* is a totally obligate apomict, the subtle balance between amphimictic and apomictic reproduction in variable species complexes cannot apply to this species. *C. jubata*, like *Calamagrostis chalybaea*, has no possibility of sporadic or local sexuality. *C. jubata* is indeed at an evolutionary dead-end. Another almost completely obligate apomictic grass, *Poa nervosa*, produces no functional pollen over most of its range (Grun, 1955), but

it is not totally unable to exchange genes with other species or races. In *C. jubata* seed-set is well advanced before anthesis so that no pollen can reach the stigmas in time to be effective. In *P. nervosa*, however, the spikelets are exposed before embryogenesis commences, so that the style can receive pollen of related species which may produce fertile hybrids. Similarly, in one area, where functional pollen is produced, eggs can be fertilized. In such areas morphological variation is considerable. *P. nervosa*, therefore, represents a condition intermediate between the extreme of *C. jubata* and the much more usual situation among grass apomicts where pollen is functional and some sexuality occurs. *Calamagrostis purpurea* and *Nardus stricta* are rather similar to *P. nervosa* since pollen-producing races are known (Nygren, 1946; Rychlewski, 1961), and in *C. lapponica* pollen production is much more general, though the apomicts are still non-pseudogamous.

When other apomictic species of *Cortaderia* are more completely known, it may prove possible to deduce how the condition in *C. jubata* has been evolved. At present it can only be supposed that gynodioecy, which is prevalent in the genus, has been followed by the suppression of hermaphrodite plants (Connor, 1974) accompanied by the adoption of apomixis. But even after this stage, considerable further evolution must have occurred in *C. jubata* to advance the time of embryo development. It may be significant, as indicating a disposition to apomixis within the Arundineae, that *Phragmites communis*, a member of another genus of this small Tribe, is mainly vegetatively propagated over at least a part of its range, the spikelets setting seed infrequently

(Luther, 1950).

Apart from the characteristic features of its apomictic mechanism, there are some aspects of the embryology of *Cortaderia* that are of interest when compared with other grasses. The tenuinucellate condition is not at all uncommon in the Gramineae, but it is not the most prevalent (Davis, 1966). It is therefore, of interest to note its presence in the Tribe Arundineae. The structure of the embryo of *Cortaderia* is typical of the Arundineae, a Tribe which in this particular character approaches the Panicoid rather than the Festucoid groups (Reeder, 1957).

The most outstanding feature of the *Cortaderia* embryo sac is the projection of haustoria from the synergids. The projection of the synergids or of the embryo sac itself into or through the micropyle is rather uncommon in Angiosperms generally (McLean and Ivimey-Cook, 1956; Davis, 1966) and no report of this among the Gramineae is known to me. Emery and Brown (1957) described and figured embryo sacs of the South African grass *Fingerhouthia africana* which protruded through the micropyle carrying the egg apparatus and polar nuclei into the ovarial cavity. Embryo and endosperm subsequently developed in the extruded part of the embryo sac. This curious structure developed in six out of twelve mature ovules investigated, but its significance is unknown. In any event it bears no resemblance to the haustorial synergids of *Cortaderia*. The occurrence of synergid haustoria in all species of *Cortaderia* investigated, suggests that this feature will prove of systematic interest, and that related genera should be examined for its presence. Since the haustoria completely

fill the micropyle, they must affect the process of fertilization. It would be of interest to observe the path taken by the pollen tube and male gametes.

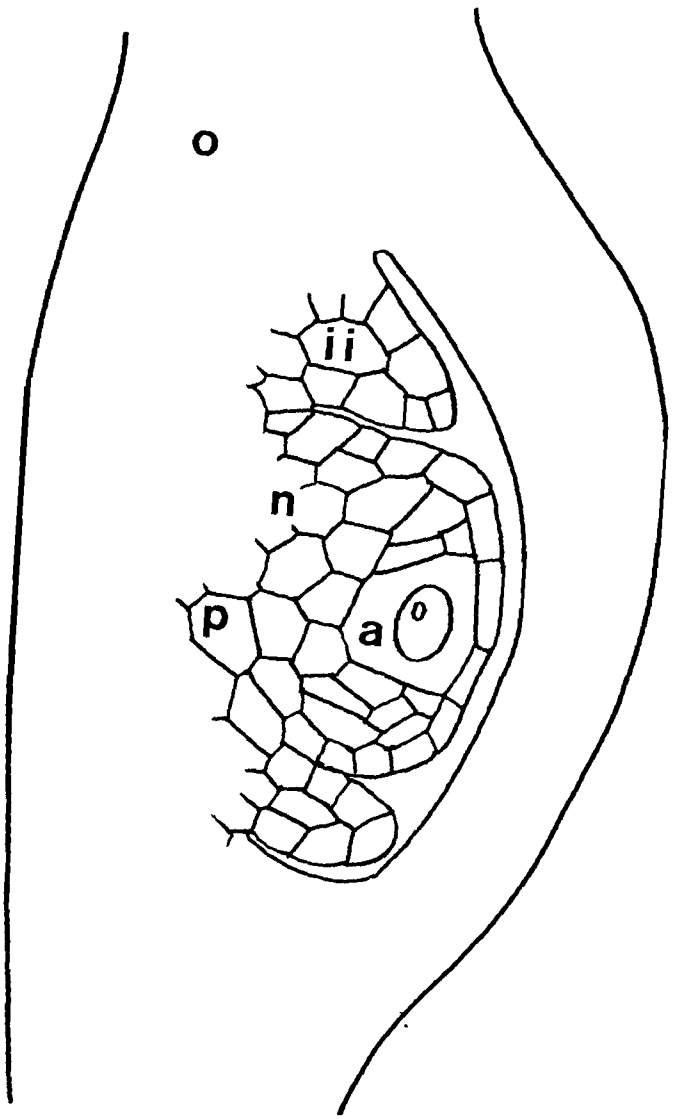
ADDENDUM

During the final stages of the preparation of this thesis, flowering material became available of *Cortaderia rudiuscula* and *Lamprothyrsus peruvianus*. Preliminary microscopic observations on them confirmed the finding of H.E. Connor that both are apomictic. *C. rudiuscula* appears to closely resemble *C. jubata* in exhibiting a number of nucellar embryo sacs, synergids with large haustorial extensions and well developed wall ingrowths over the internal surface of the embryo sac. It is non-pseudogamous. *Lamprothyrsus peruvianus* is also non-pseudogamous, but preliminary investigations are not far enough advanced to indicate whether the embryo sac is somatic or gonial in origin.

Fig. 3

Ovary in LS. Young ovule with archesporial cell and inner integument. X 850.

a	archesporial cell
ii	inner integument
n	nucellus
o	ovary
p	placenta

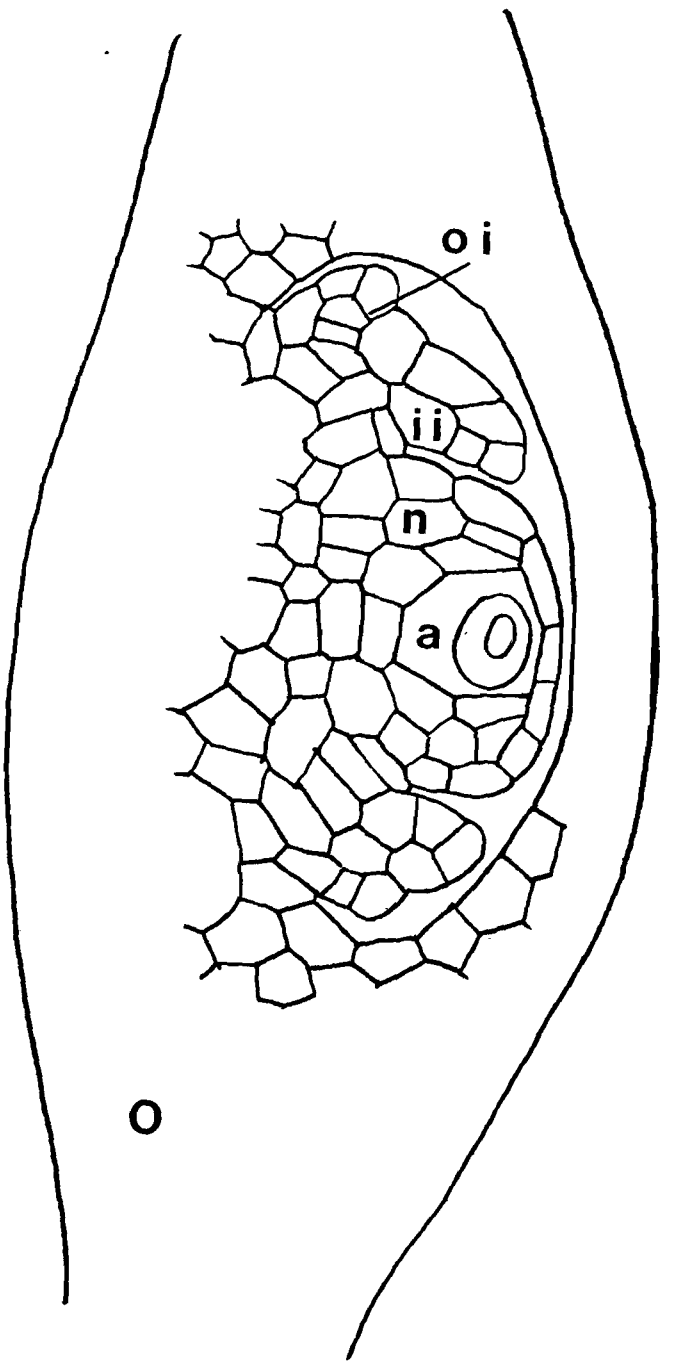


3

Fig. 4

Ovary in LS. Young ovule with archesporial cell and showing initiation of outer integument. X 850.

a	archesporial cell
ii	inner integument
n	nucellus
o	ovary
oi	outer integument

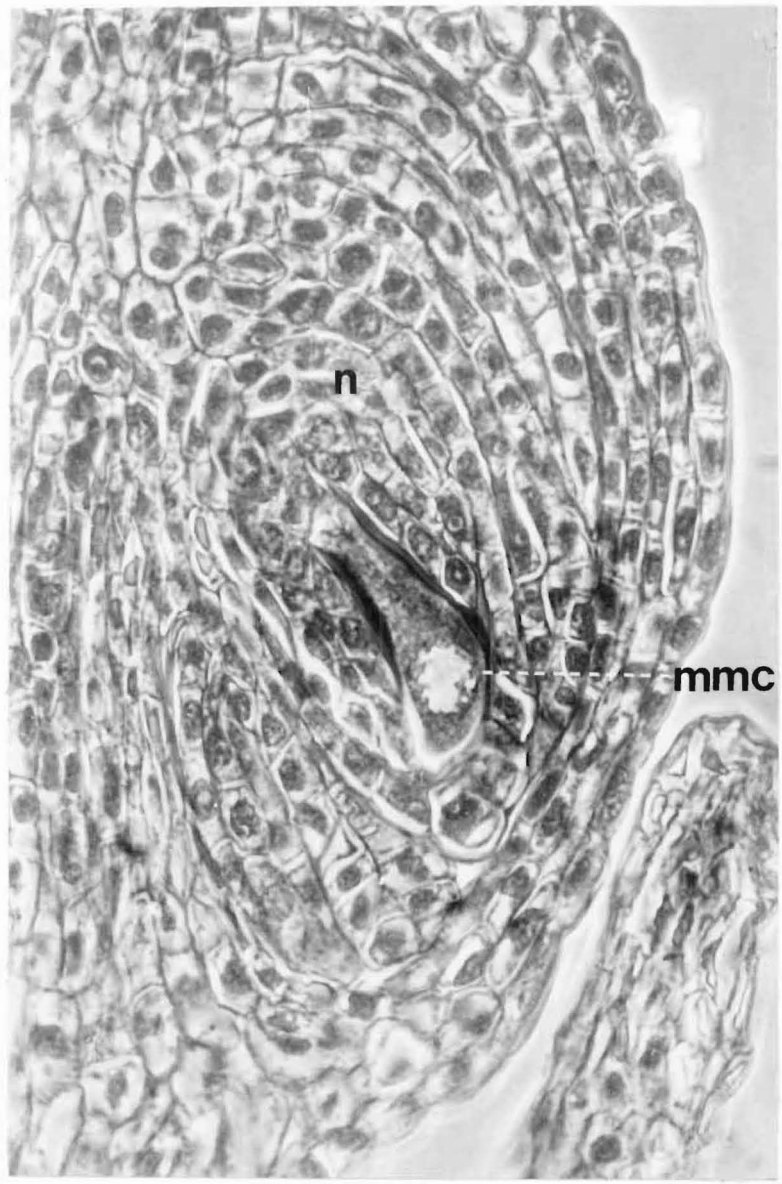


4

Fig. 5

Young ovule in LS. The nucellus contains a mature megaspore mother cell. X 650.

mmc	megaspore mother cell
n	nucellus

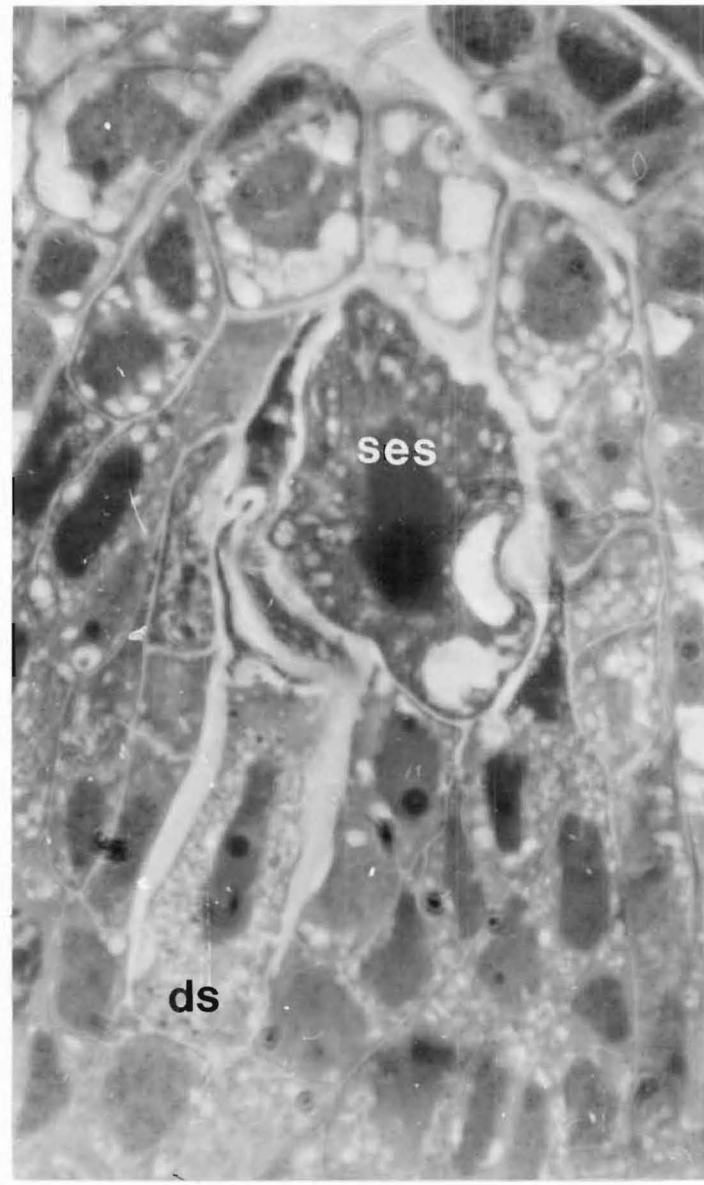


5

Fig. 6

Young ovule in LS. The nucellus contains a degenerating spore with the remains of other spores above it. An adjacent enlarging nucellar cell still contains only one nucleus. X 1500.

ds degenerating spore
ses somatic embryo sac

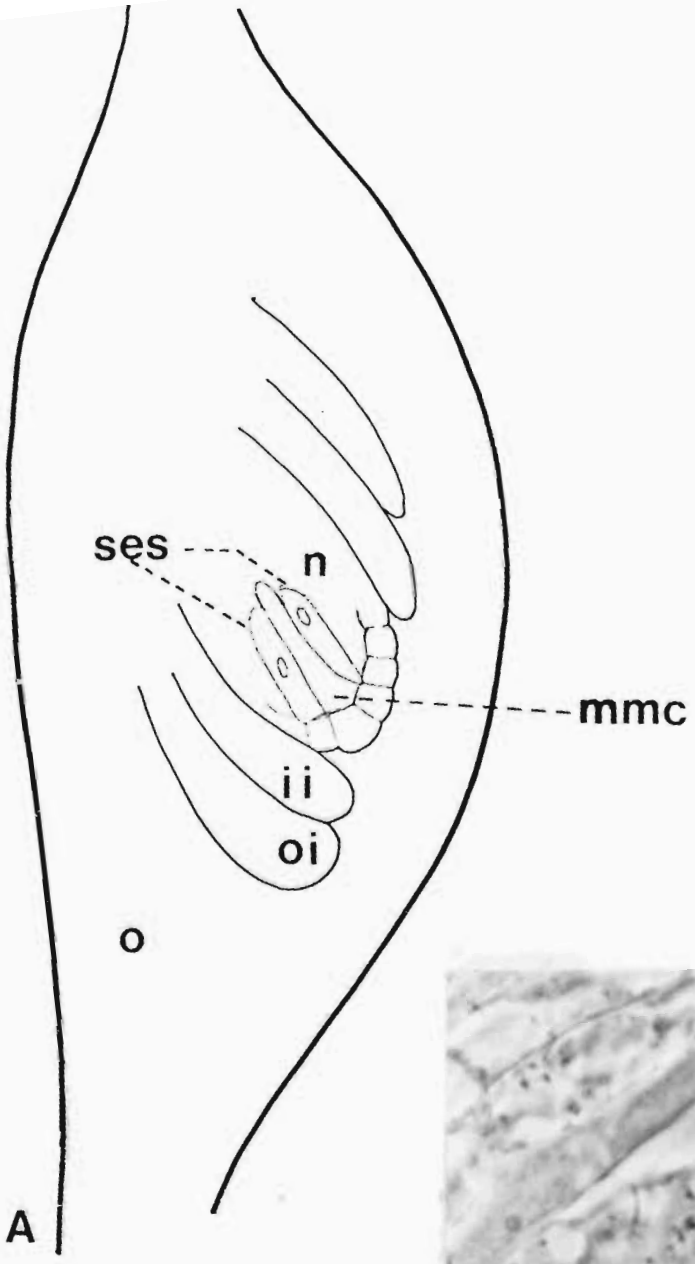


6

Fig. 7

Ovary in LS. Nucellus with degenerating megaspore mother cell and enlarging nucellar cells.
A, outline drawing of whole ovary, X 400.
B, the apex of the nucellus (phase optics), X 1700.

ii	inner integument
mmc	megaspore mother cell
o	ovary
oi	outer integument
ses	somatic embryo sac



A

7

B

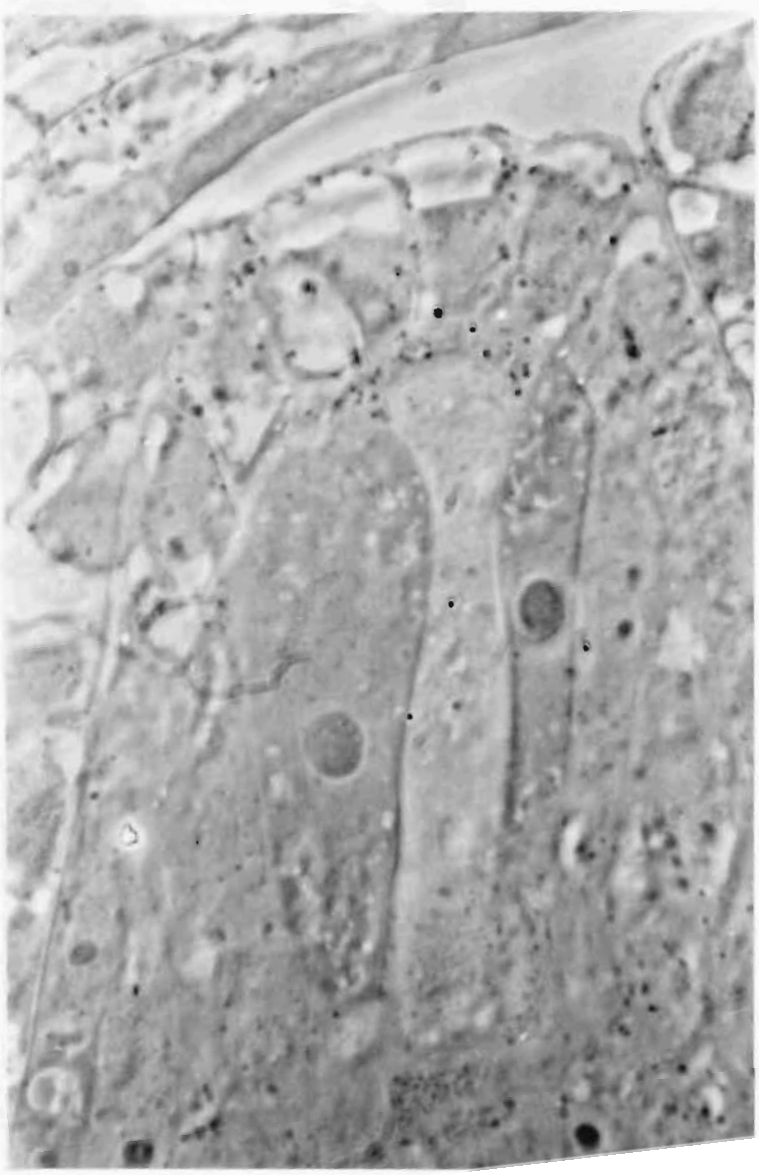
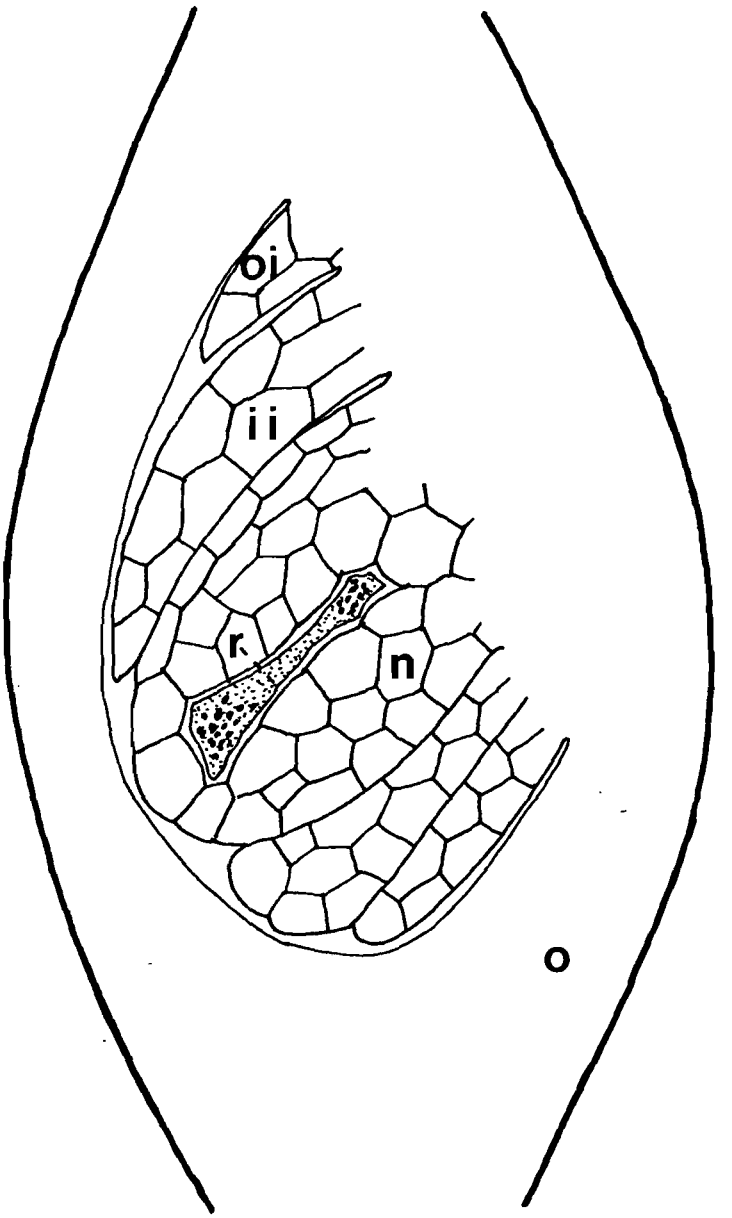


Fig. 8

Ovary in LS. Nucellus with degenerate megaspore mother cell. X 730.

ii	inner integument
n	nucellus
o	ovary
oi	outer integument
r	remains of megaspore mother cell

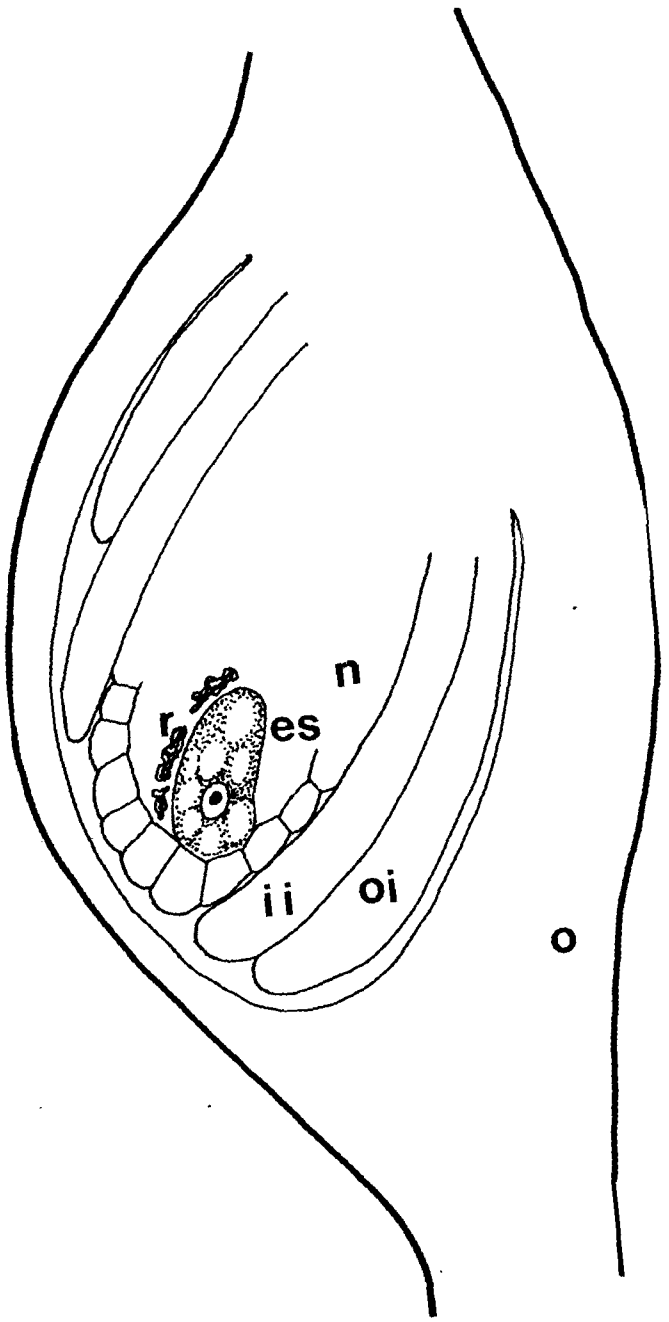


8

Fig. 9

Ovary in LS. Nucellus contains remnants of the megaspore mother cell (or its derivatives) and an enlarging nucellar cell (= somatic embryo sac). The micropyle is not yet formed. X 600.

es	somatic embryo sac
ii	inner integument
n	nucellus
o	ovary
oi	outer integument
r	remnants of megaspore mother cell

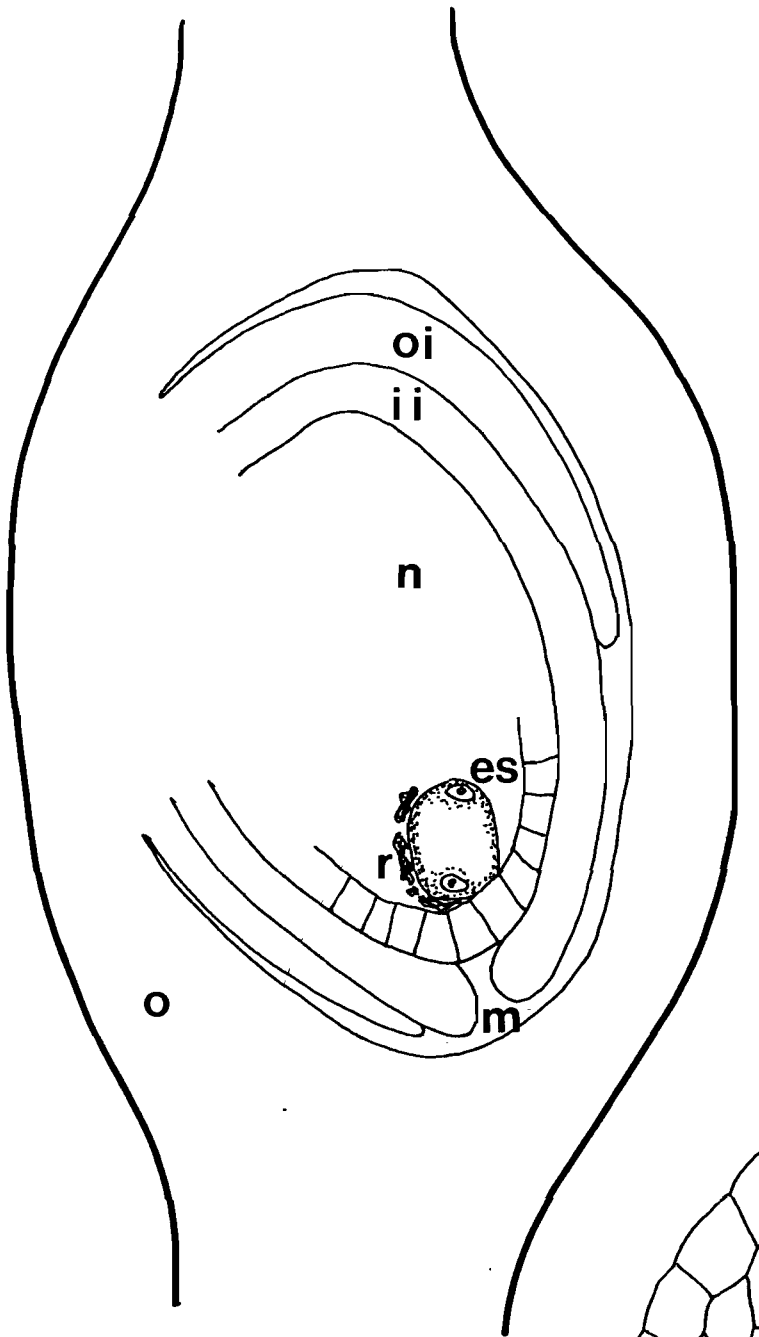


9

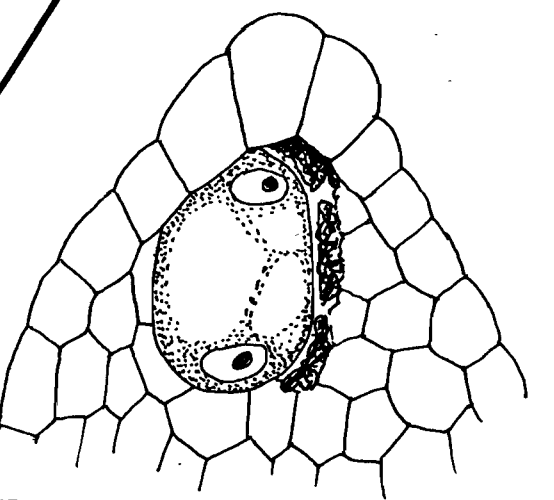
Fig. 10

Ovary in LS. Nucellus curving towards base of ovary, micropyle formed by inner integument.
A, 2-nucleate somatic embryo sac with adjacent disintegrated megaspore mother cell. X 400.
B, detail of nucellar apex. X 800.

es	embryo sac
ii	inner integument
m	micropyle
n	nucellus
o	ovary
oi	outer integument
r	remains of megaspore mother cell



A



B

Fig. 11

Ovary in LS. The ovule contains two somatic embryo sacs, one with four nuclei. X 375.

es	embryo sac
ii	inner integument
m	micropyle
n	nucellus
oi	outer integument

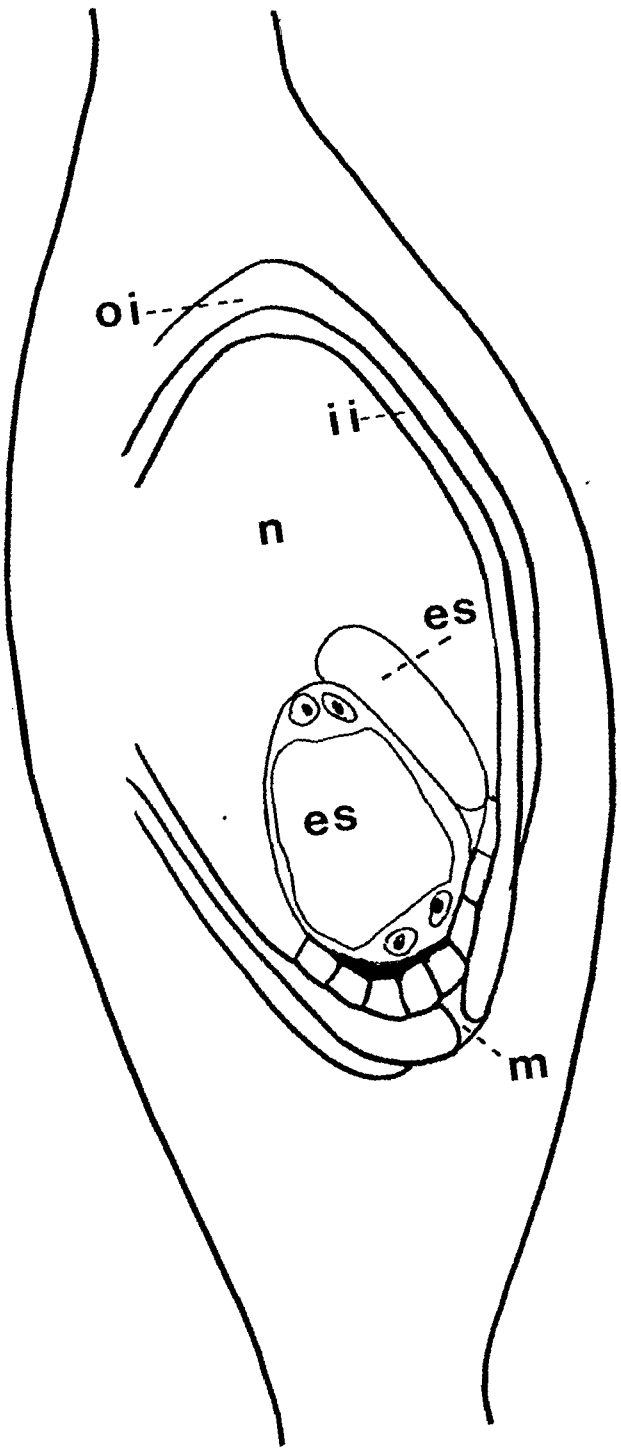
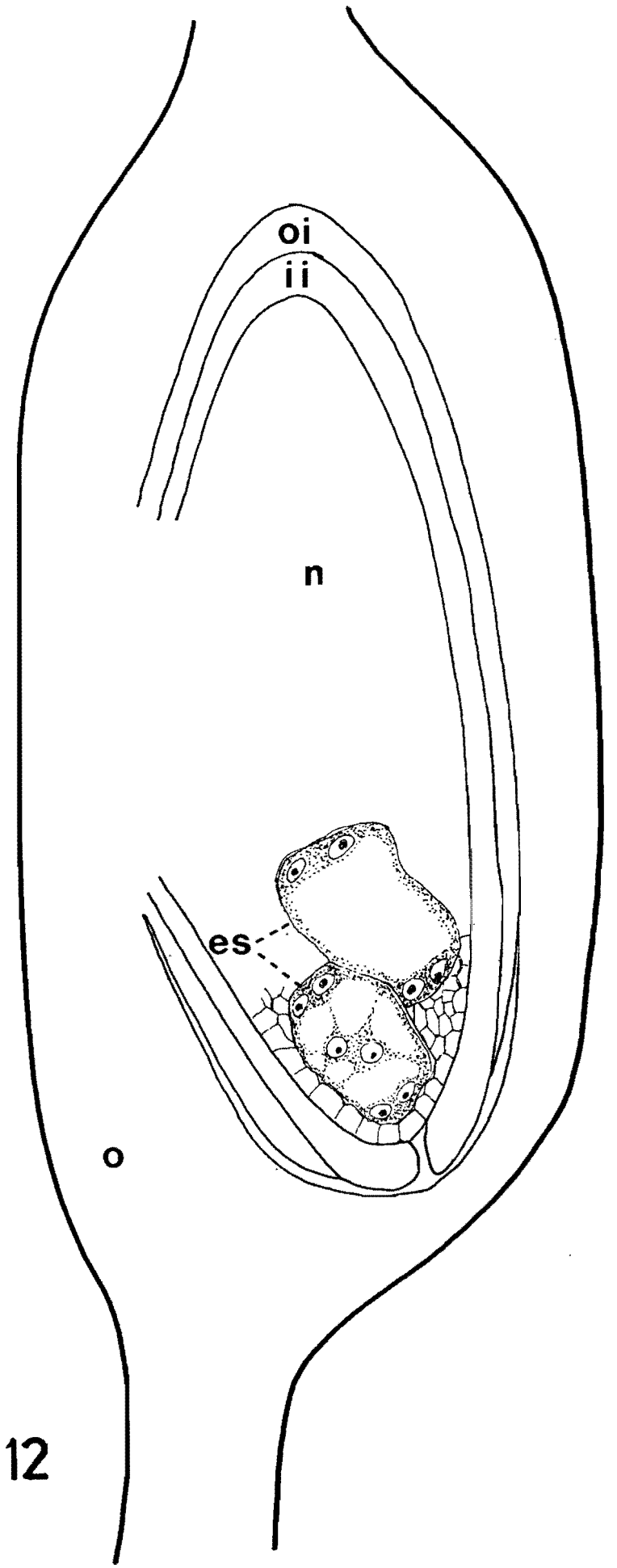

oi
ii
n
es
es
m

Fig. 12

Ovary in LS. Nucellus with two somatic embryo sacs, one 6-nucleate and becoming organized, the other 4-nucleate. X 400.

es	embryo sac
ii	inner integument
n	nucellus
o	ovary
oi	outer integument

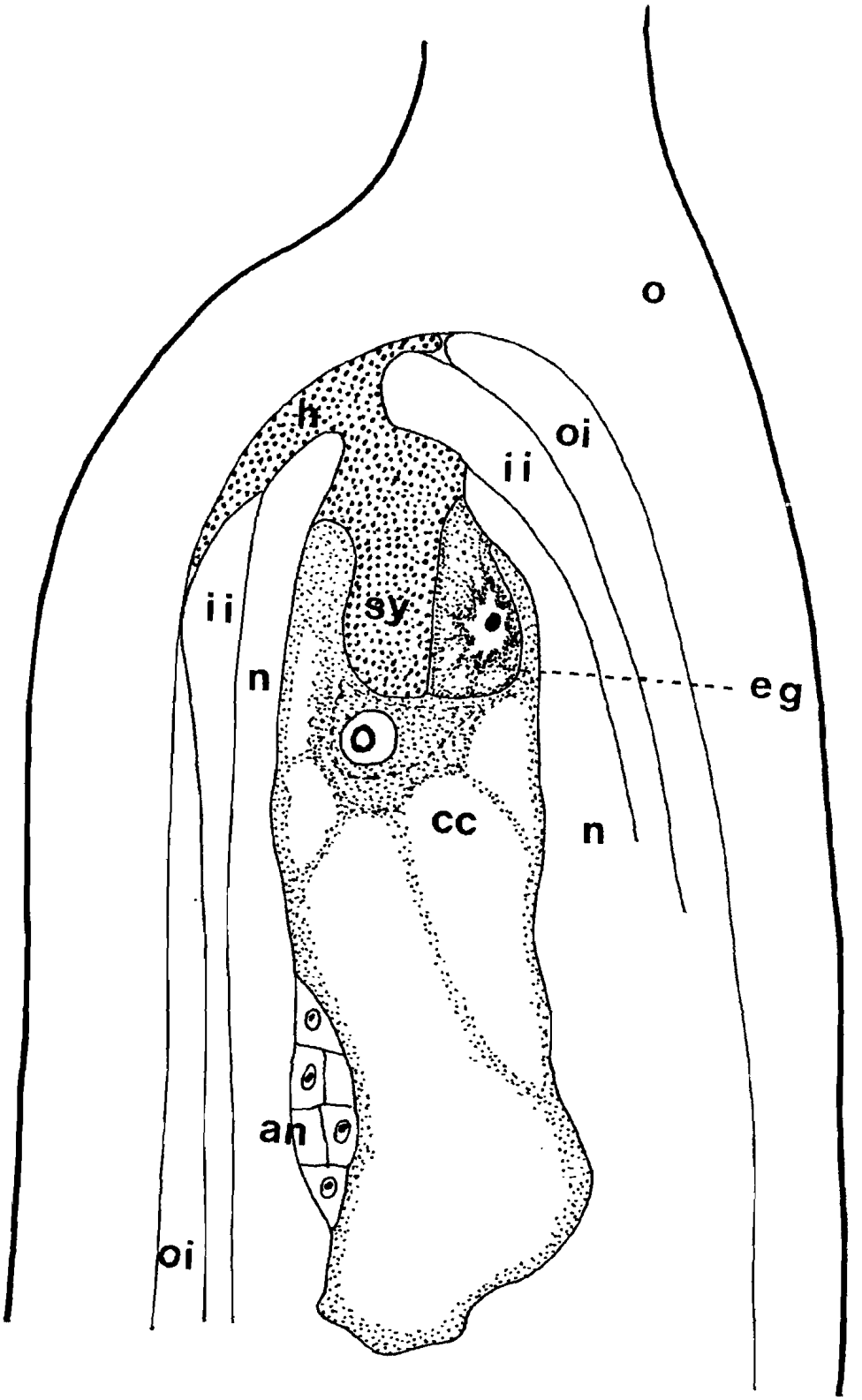


12

Fig. 13

Ovary in LS. Ovule with mature embryo sac. X 525.

an	antipodal cells
cc	central cell
eg	egg cell
h	haustorium
ii	inner integument
n	nucellus
o	ovary
oi	outer integument
sy	synergid

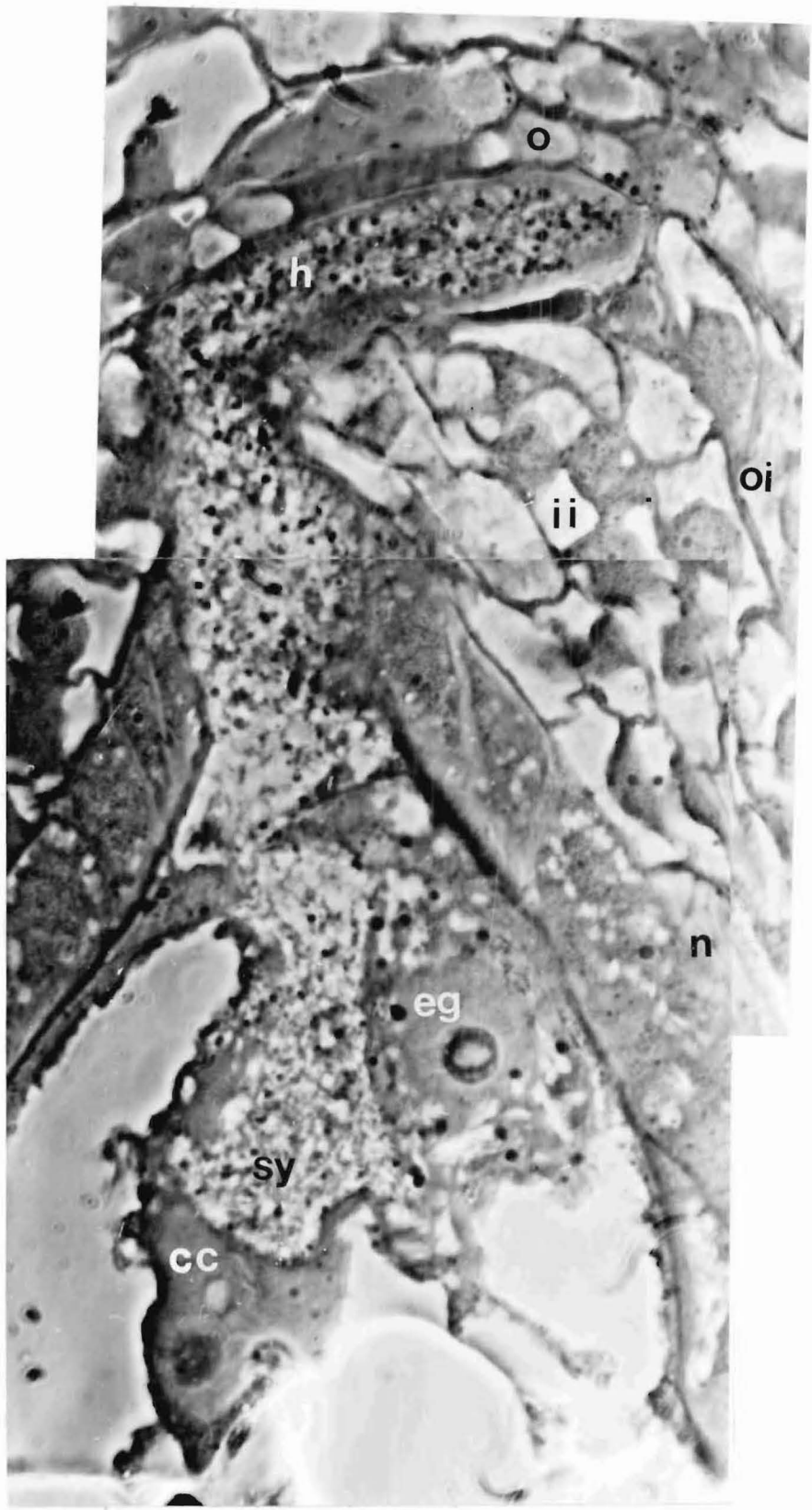


13

Fig. 14

Details of the micropylar region of mature embryo sac.
A, LS through egg nucleus, central cell nucleus, synergid and haustorium.
B, median LS of haustorium. Both X 1100.

cc	central cell nucleus
eg	egg cell nucleus
h	haustorium
ii	inner integument
n	nucellus
o	ovary
oi	outer integument
sy	synergid



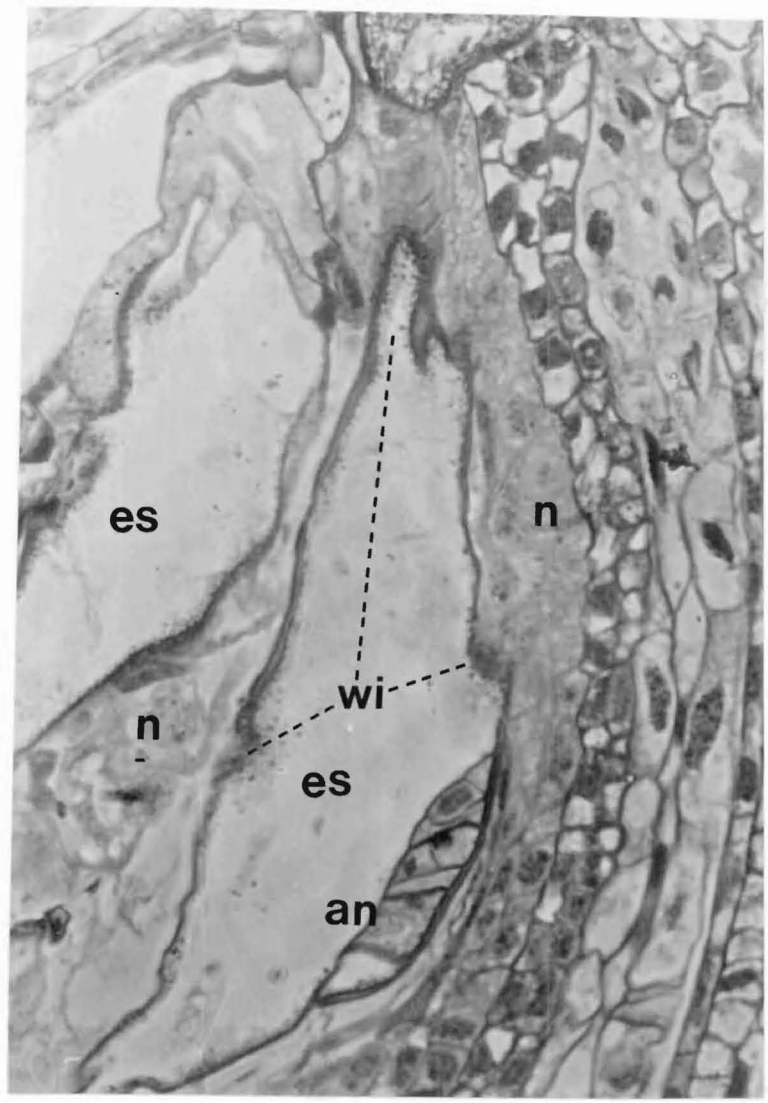
14 A

14 B

Fig. 15

LS passing through two somatic embryo sacs, one with a lateral group of antipodal cells. Conspicuous wall ingrowths occur on all parts of the embryo sac walls. X 550.

an	antipodal cells
es	embryo sac
ii	inner integument
n	nucellus
wi	wall ingrowths



15

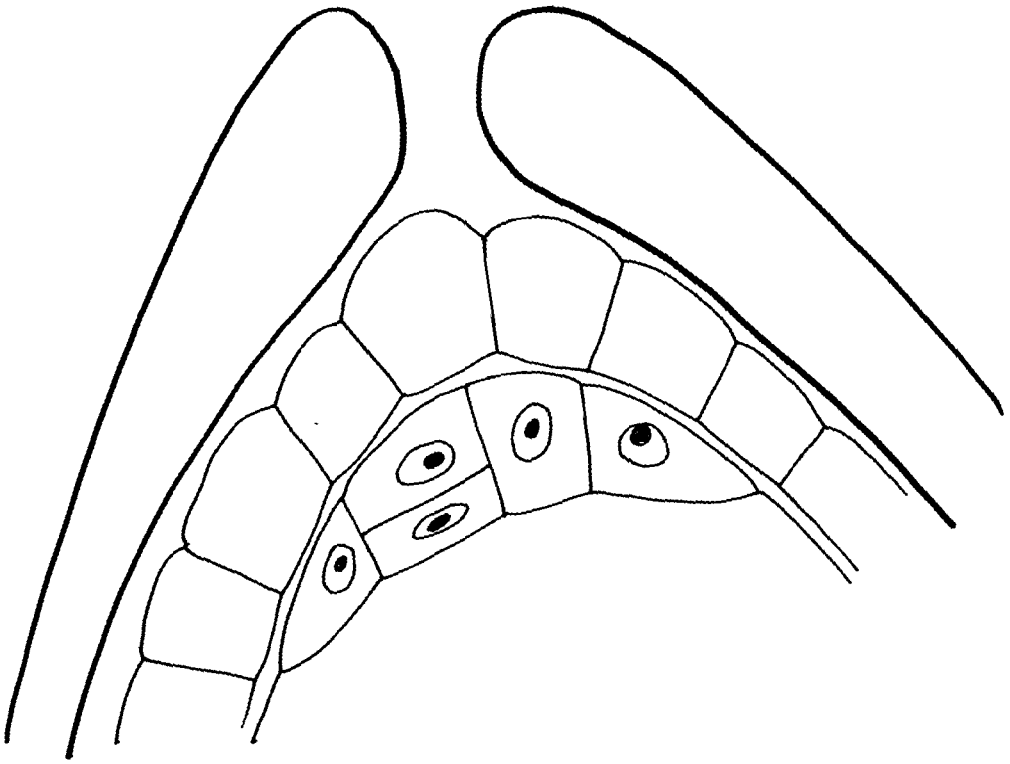
Fig. 16

Group of antipodal cells at the micropylar end of an inverted embryo sac. X 1000.

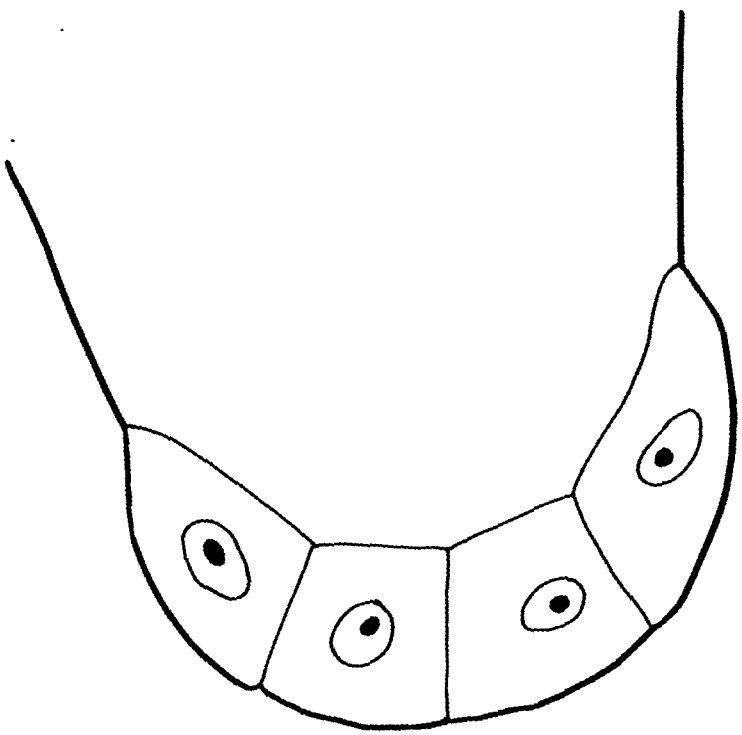
Fig. 17

Group of antipodal cells in a subspherical chalazal caecum. X 1000.

an	antipodal cells
ii	inner integument
m	micropyle
n	nucellus



16



17

Fig. 18

LS through the upper part of an ovule with one normally developed somatic embryo sac near the micropyle, and a second atypical embryo sac towards the chalazal end. Normal components cannot be certainly recognized in this second embryo sac, but two polar nuclei occupy a central position, and one of three cells at the micropylar end has developed an haustorium. X 400.

aes	abnormal embryo sac
es	typical somatic embryo sac
h	haustorium

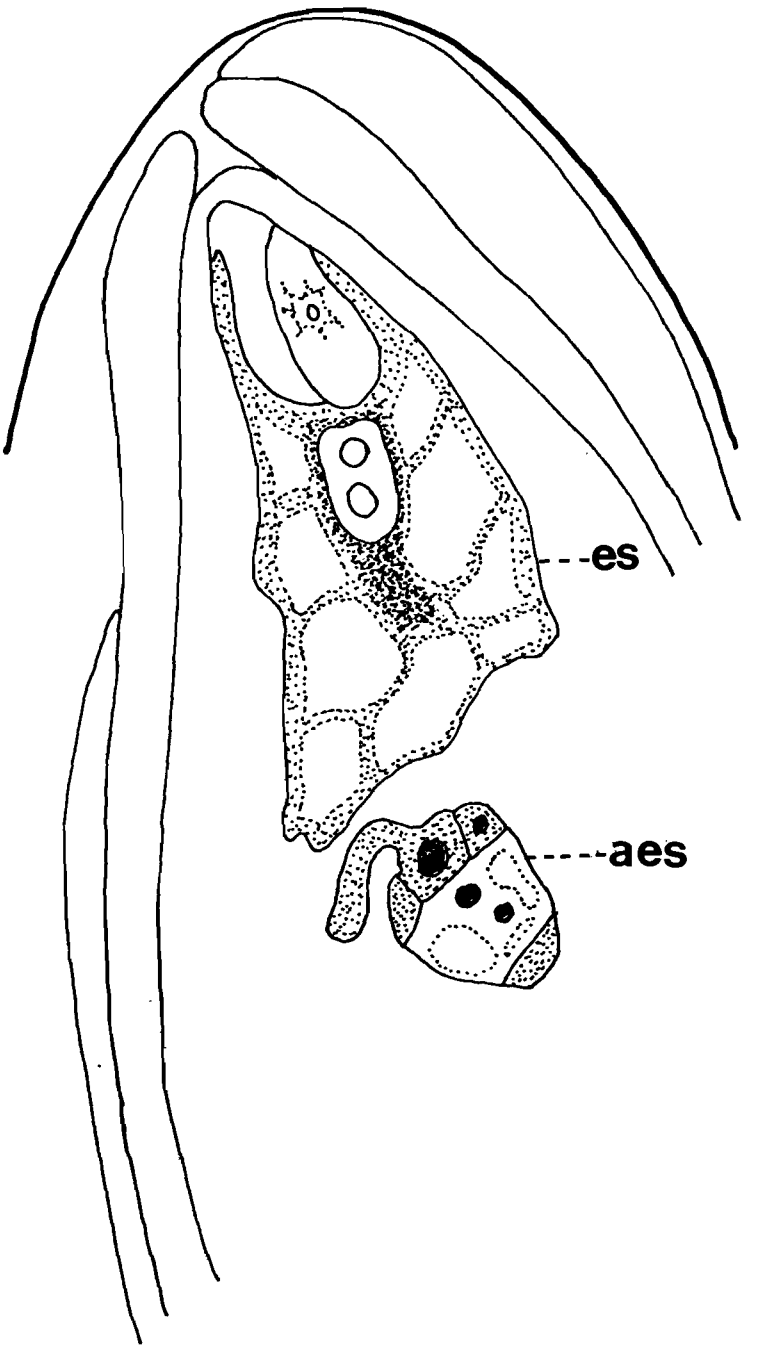

es
aes

Fig. 19

Embryo sac with egg cell prolonged as a micropylar haustorium. The haustorium of the synergid crosses behind that of the egg. X 360.

cc	central cell
eg	egg cell
h	haustorium
ii	inner integument
n	nucellus
pn	polar nuclei
sy	synergid

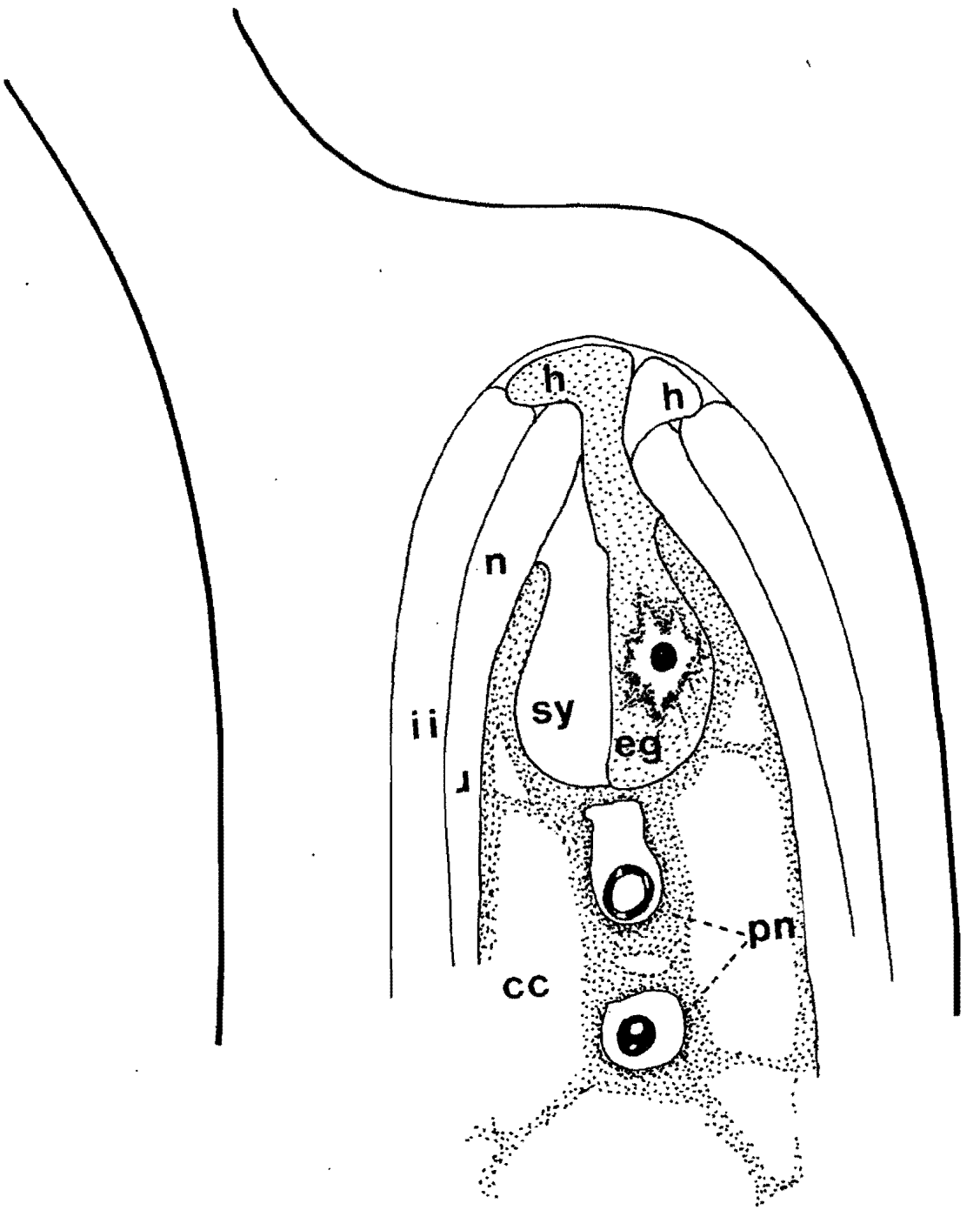
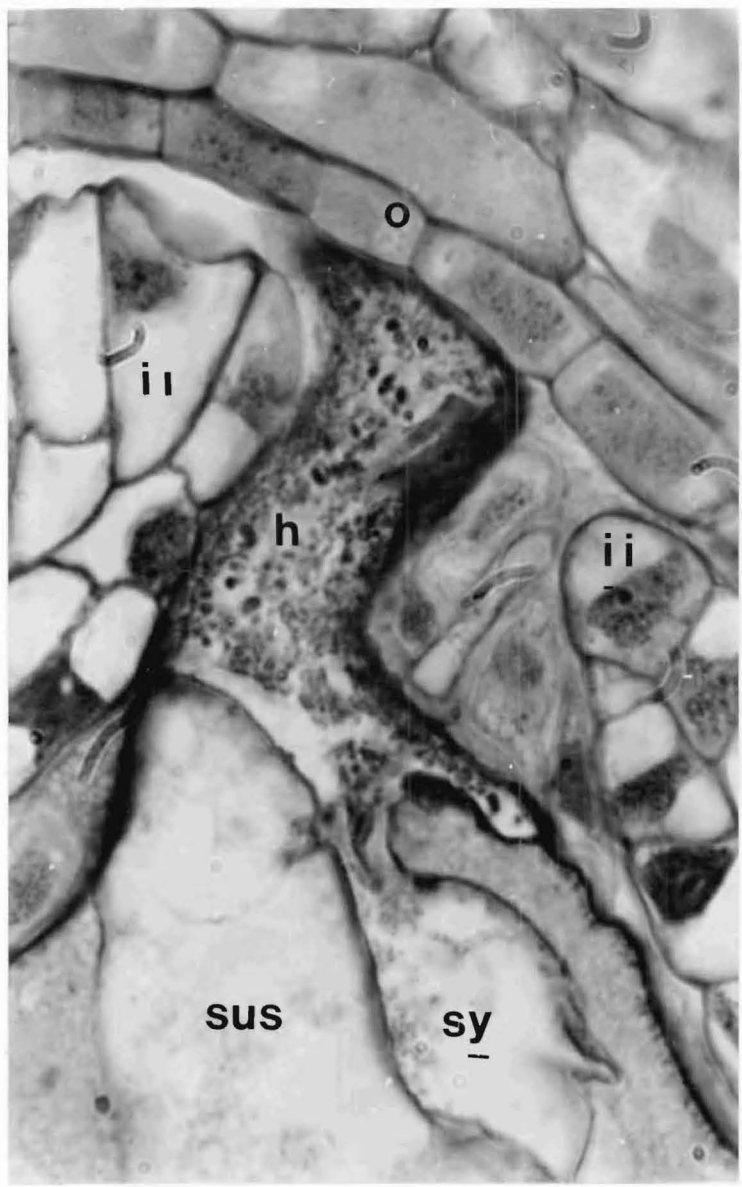

h
h
n
ii
sy
eg
pn
cc

Fig. 20

LS through the micropylar region of an embryo sac containing an embryo at the globular stage. The haustorium still appears active but the synergid is empty and has collapsed. X 1200.

h	haustorium
ii	inner integument
n	nucellus
o	ovary
sus	suspensor of embryo
sy	degenerate synergid

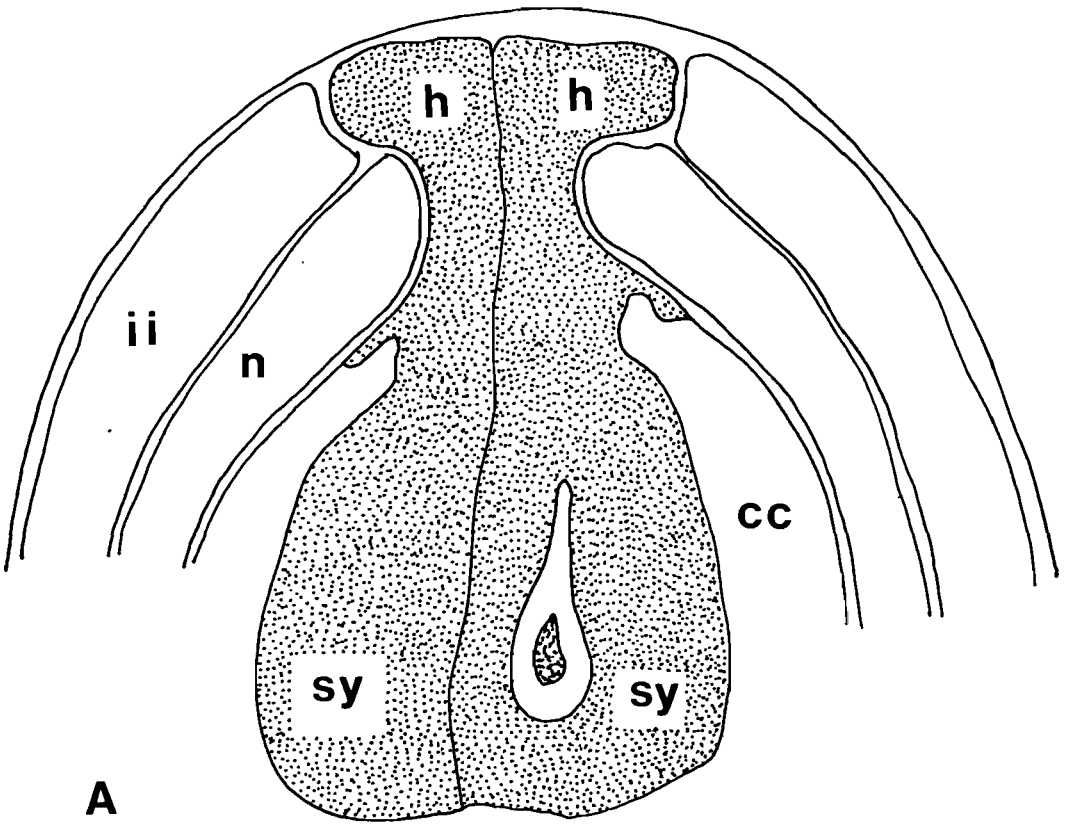


20

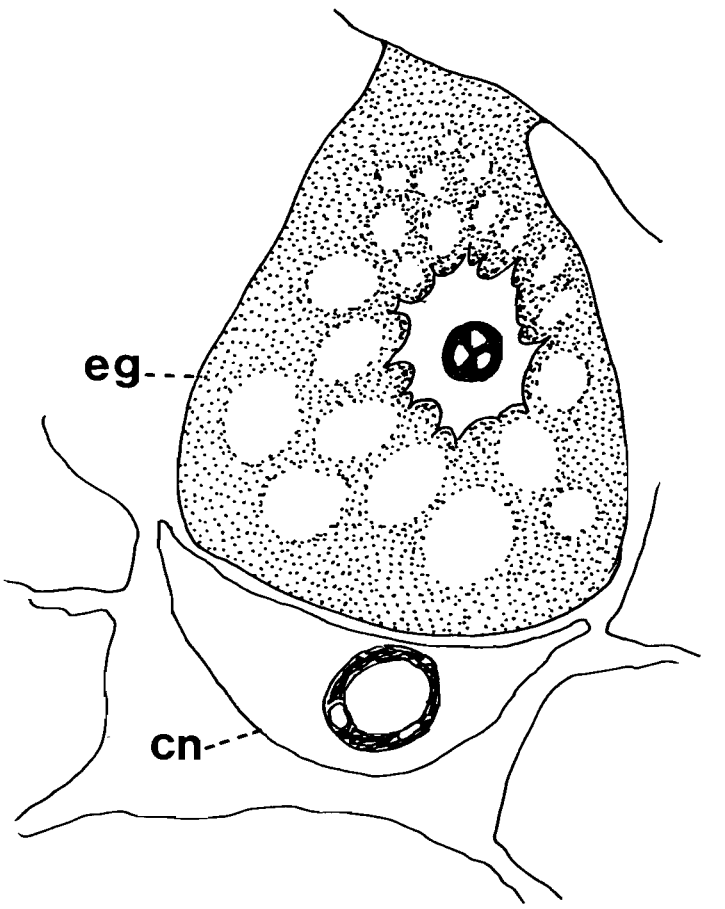
Fig. 21

A, embryo sac with two fully developed synergids, each with an haustorium.
B, egg cell and central cell (from another section of the same series).
Both X 1300.

cc	central cell
cn	central cell nucleus
h	haustorium
ii	inner integument
n	nucellus
sy	synergid



A



B

Fig. 22

Portion of ovary in LS. Two somatic embryo sacs have developed in the ovule. In 1, the haustorium passes through the micropyle, while in 2 it penetrates the nucellus laterally. X 300.

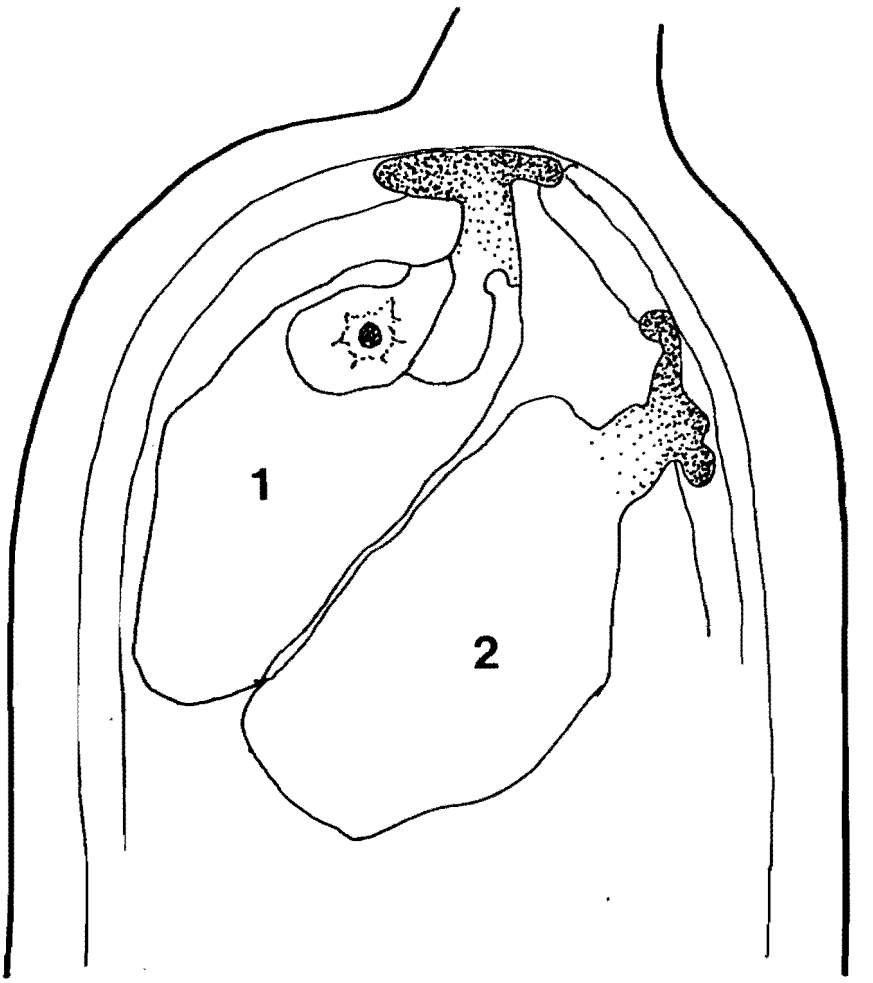

1
2

Fig. 23

Ovary in LS. Two somatic embryo sacs have developed in the ovule. In 1, the haustorium passes through the micropyle, while in 2 it penetrates the nucellus laterally. X 260.

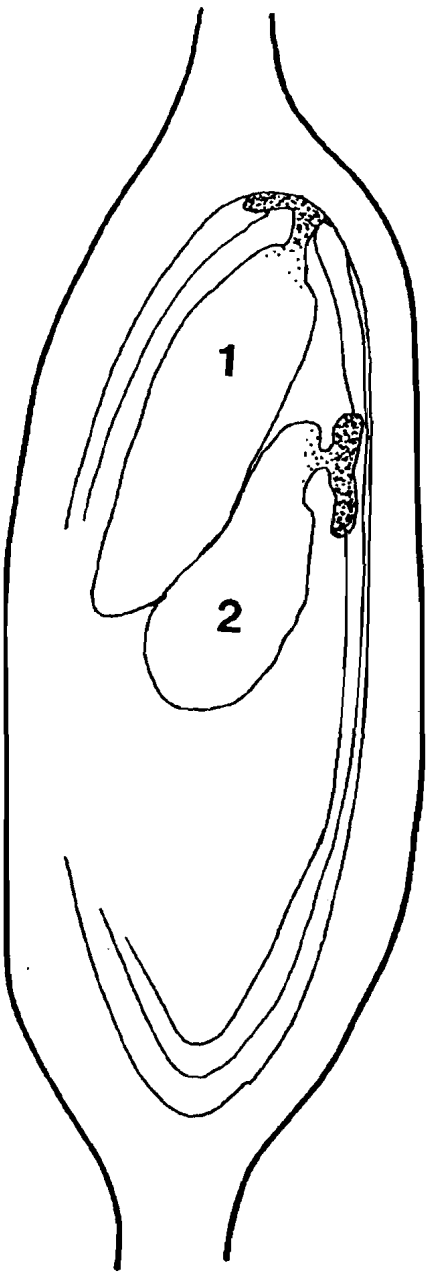

1
2

Fig. 24

Half of ovary in LS. Three embryo sacs have developed in the ovule. 1, contains a globular embryo (which is lying in the micropyle) and a group of antipodal cells. The central part of this sac is constricted but contains a few endosperm cells (not shown). 2, contains an undivided egg, a synergid with haustorium (deflected from the micropyle), two polar nuclei and a group of antipodals. 3, is empty except for a group of antipodal cells at the micropylar end, adjacent to the antipodals of the other two sacs. X 400.

an	antipodals
e	embryo
eg	egg cell
h	haustorium
ii	inner integument
m	micropyle
n	nucellus
oi	outer integument
pn	polar nuclei
sy	synergid

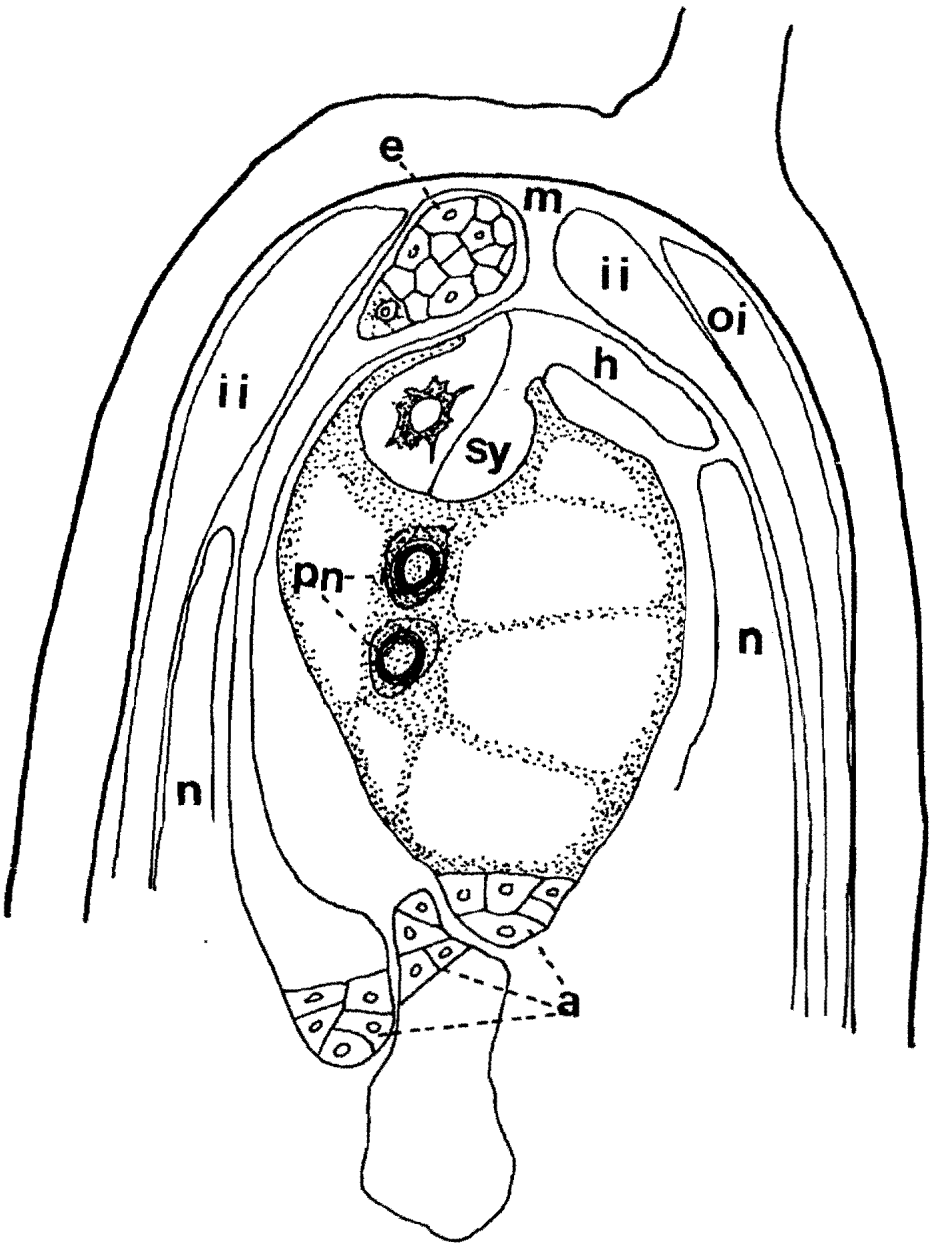
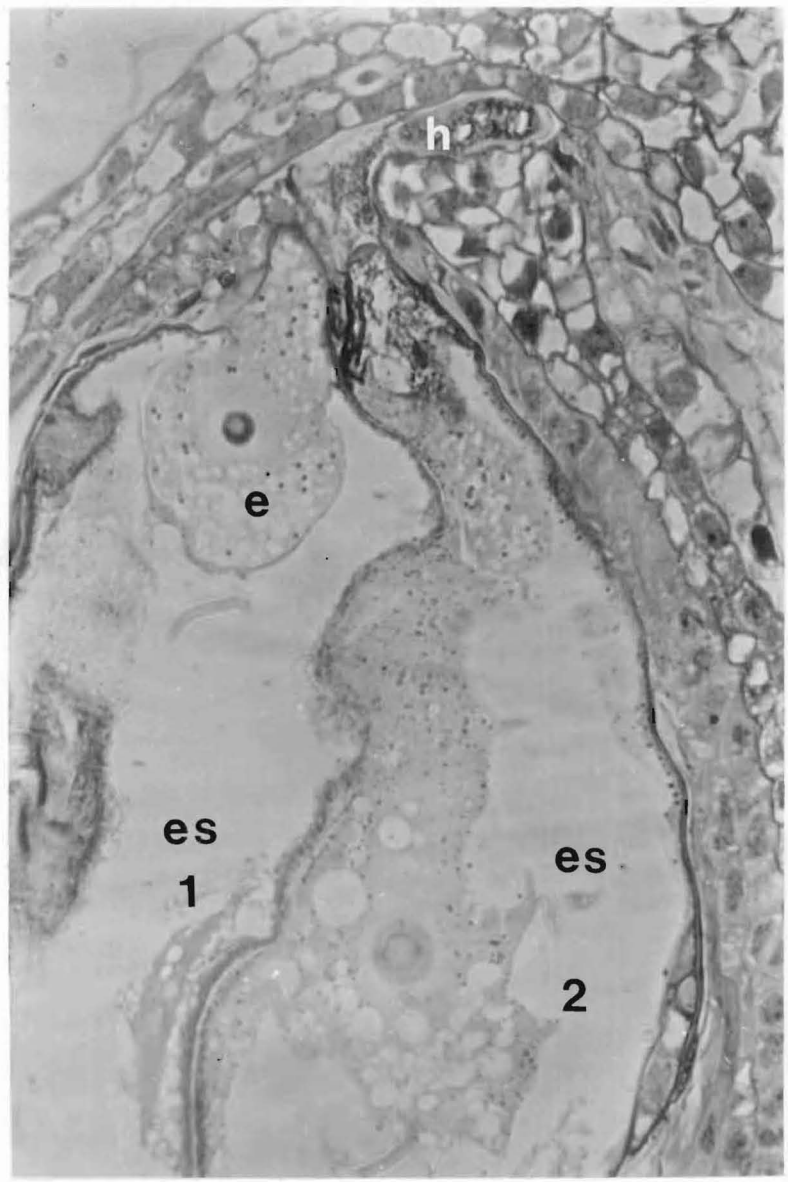

e
m
ii
oi
h
ii
sy
pn
n
n
a

Fig. 25

LS through an ovule containing two embryo sacs.
1, contains an egg cell which has just divided (the division is not visible in this section) - this embryo sac has no synergid nor haustorium.
2, contains an undivided egg. Its synergid is produced as a well developed haustorium which lies adjacent to the suspensor of the embryo in the neighbouring embryo sac. X 600.

e embryo
es embryo sac (1 and 2)
h haustorium



25

Fig. 26

LS through the upper part of an ovule. The embryo sac contains an undivided egg surrounded by peripheral coenocytic endosperm. X 660.

e	endosperm (coenocytic)
eg	egg cell
ii	inner integument
n	nucellus

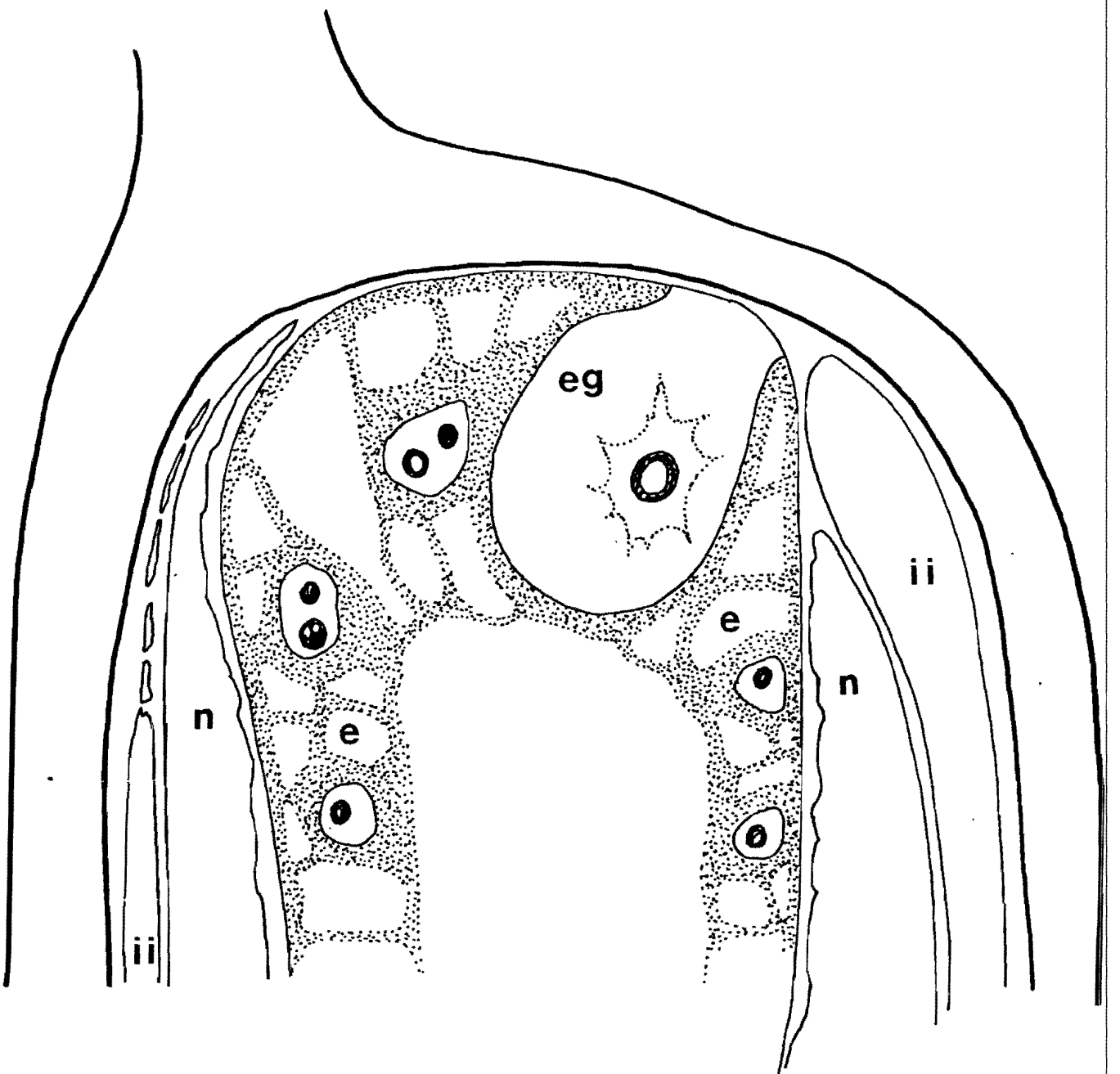

eg
ii
e
n
n
e
ii

Fig. 27

A few endosperm cells from a caryopsis with a maturing embryo. The nuclei are very irregular in shape and the cytoplasm is rich in starch grains. X 1250.

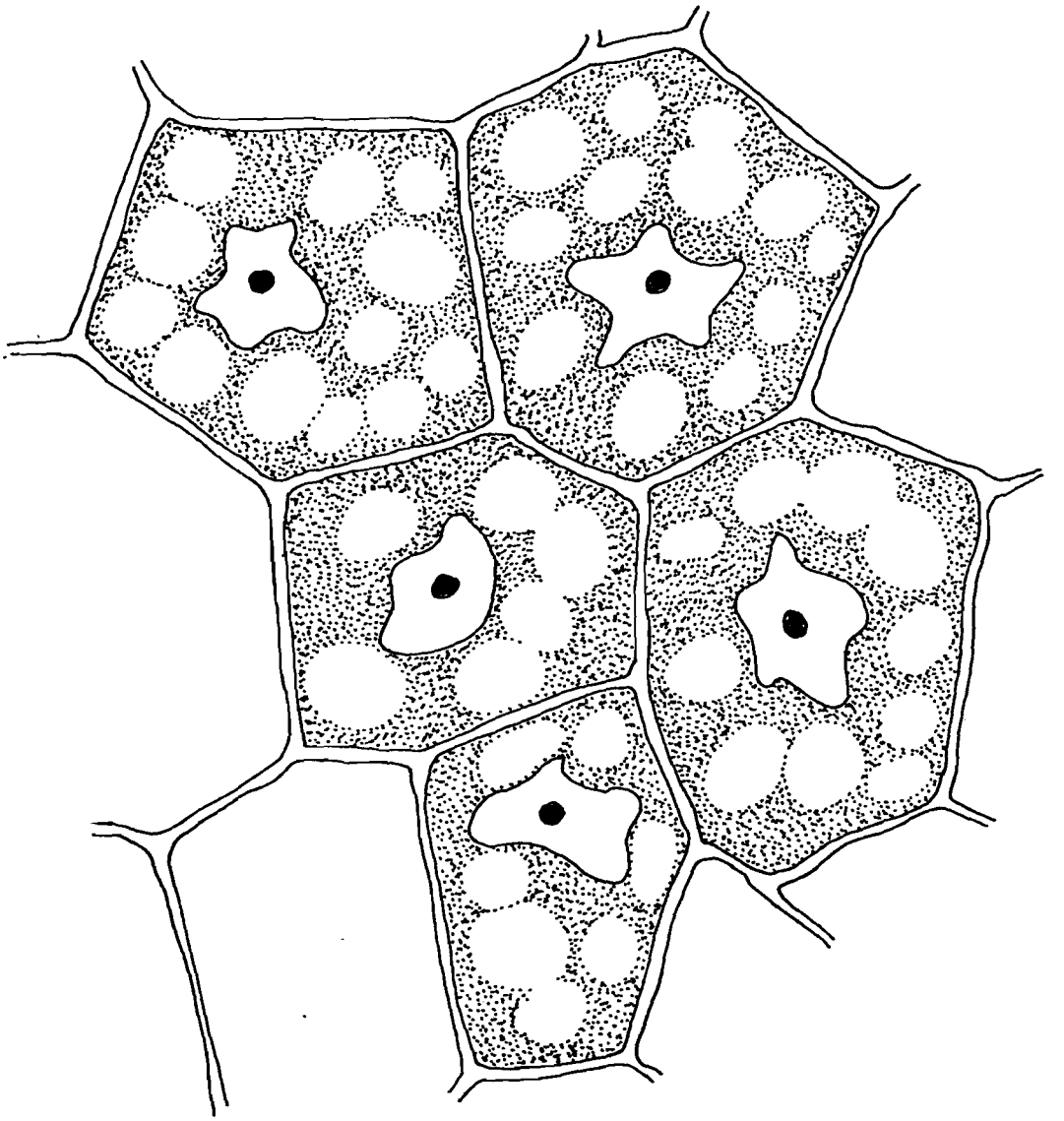
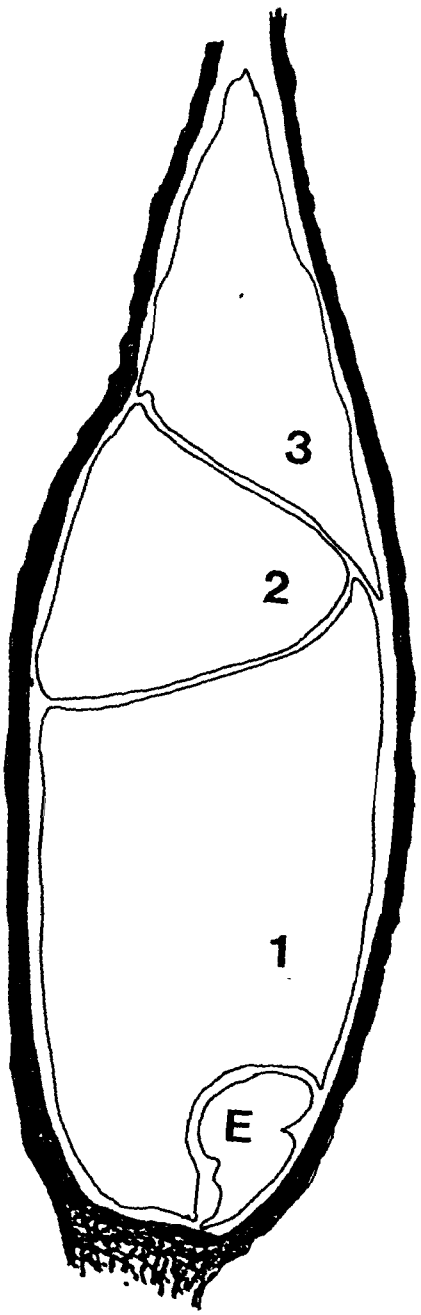


Fig. 28

Maturing caryopsis in LS, with one embryo and three zones of endosperm, each derived from a separate embryo sac. X 100.

e embryo
1,2,3 zones of endosperm

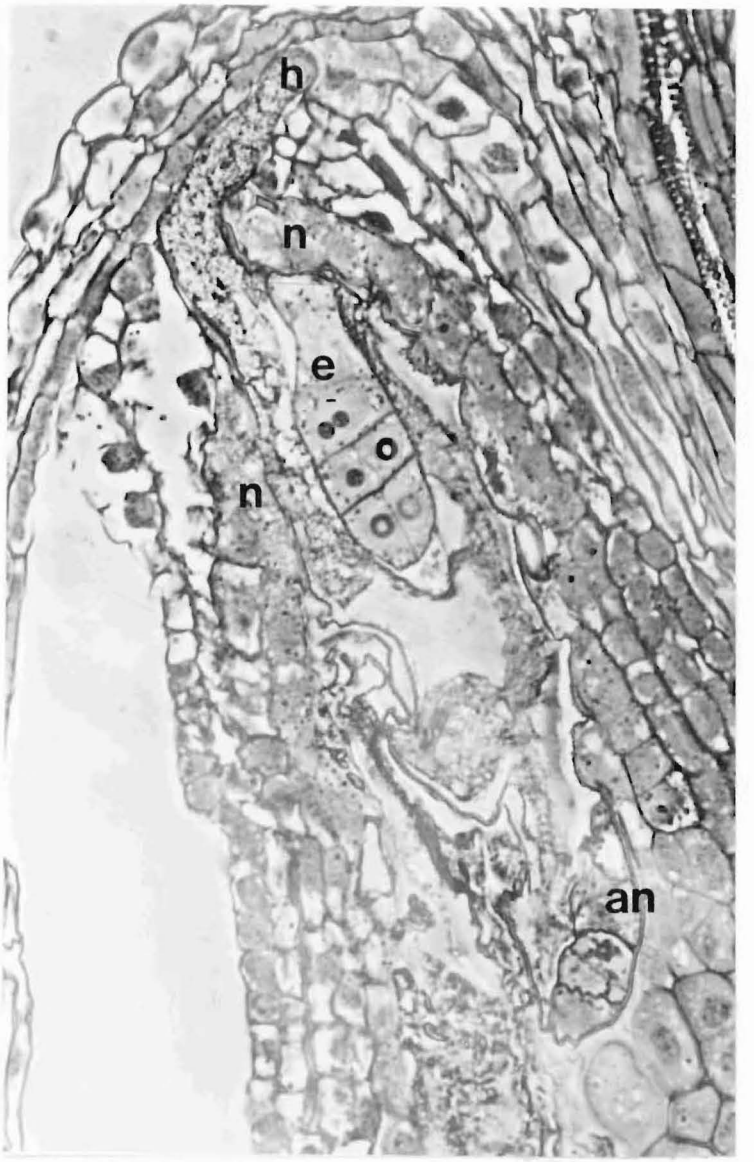


28

Fig. 29

LS through micropylar region of an ovule in which a pro-embryo is developing. The pro-embryo is attached to the wall of the embryo sac adjacent to a collapsed synergid. The haustorium still has an active appearance. The central cell nucleus is still undivided (not visible in this section) and the antipodals retain their contents. X 600.

an	antipodal cells
e	pro-embryo
h	haustorium
n	nucellus



29

Fig. 30

LS through the upper part of an ovule containing two embryo sacs each with an undivided central cell nucleus.
1, contains an undivided egg cell.
2, contains a pro-embryo.
X 600.

cn	central cell nucleus
e	pro-embryo
eg	egg cell
es	embryo sac (1 and 2)
n	nucellus

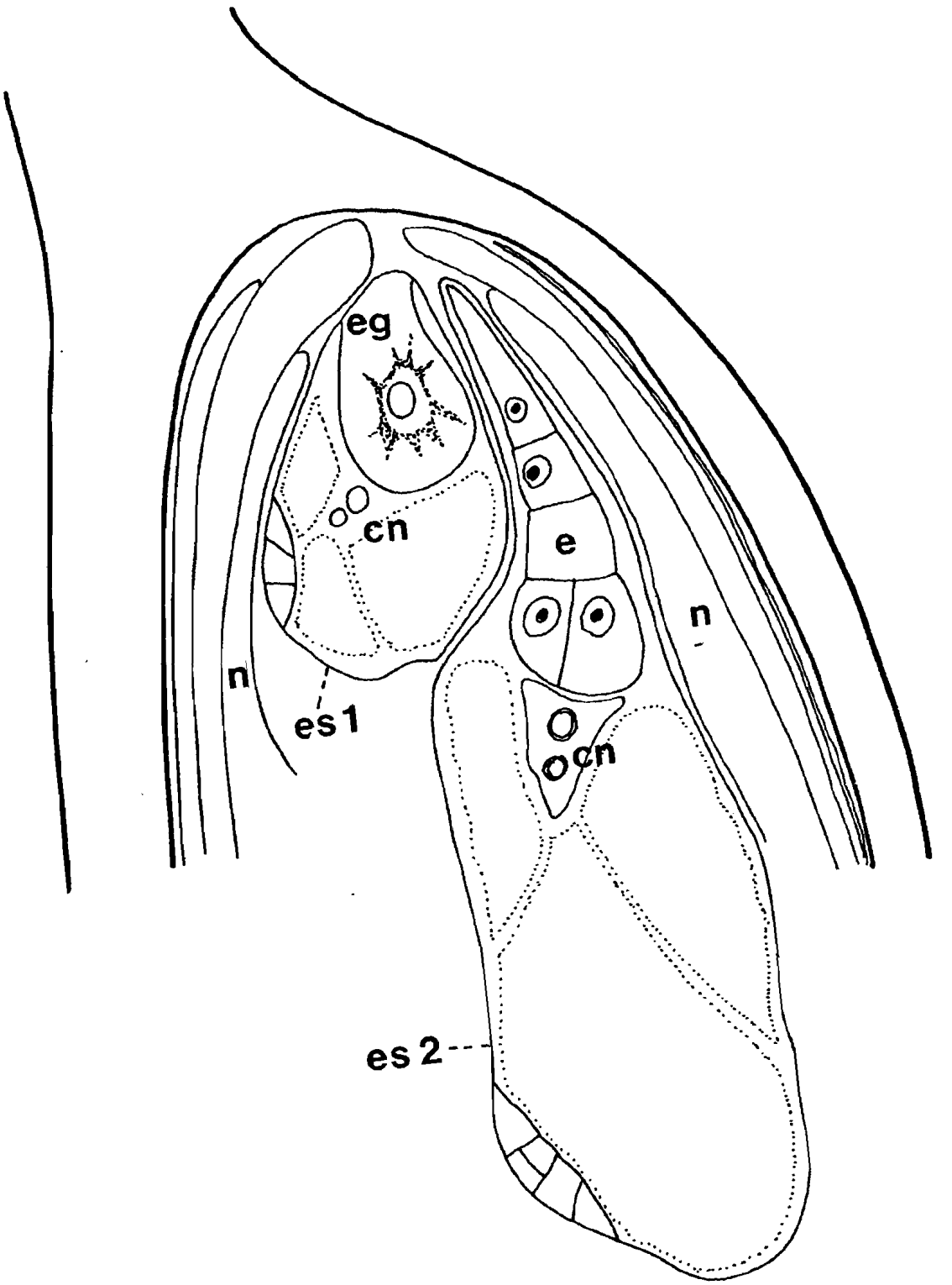

eg
cn
e
n
n
es 1
ocn
es 2

Fig. 31

Micropylar region of an ovule with a pro-embryo at a stage similar to that in Fig. 29. The central cell nucleus is undivided. X 750.

cn	central cell nucleus
e	pro-embryo
h	haustorium
n	nucellus
sy	synergid (collapsing and with few contents)

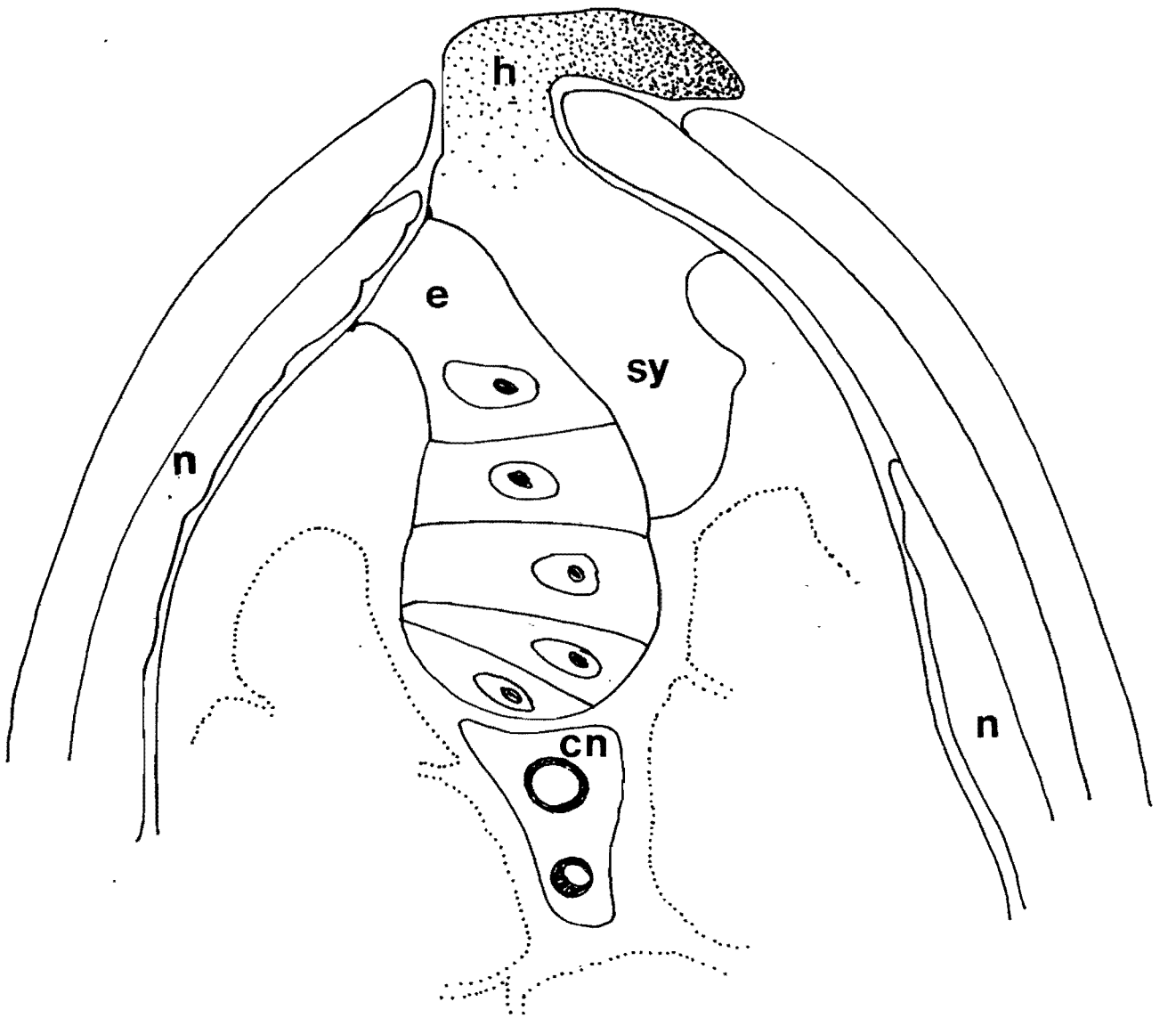
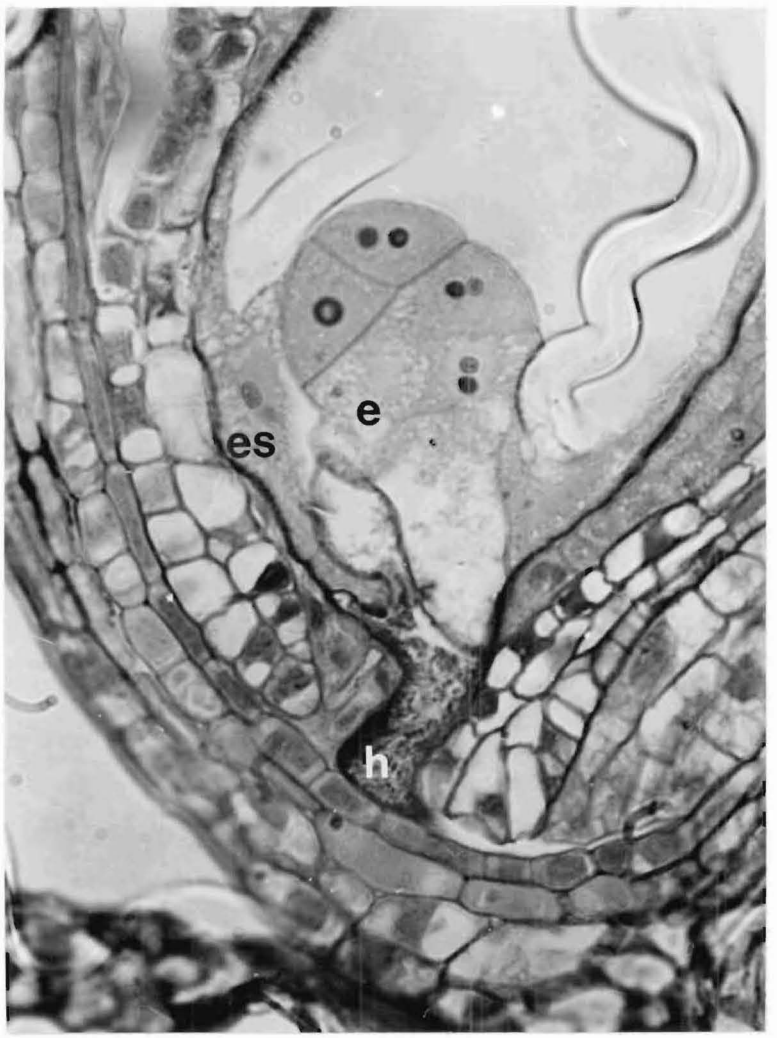

h
e
sy
n
n
cn

Fig. 32

Young embryo slightly advanced beyond that in Fig. 31 and entering the globular stage. The endosperm is still coenocytic. The synergid has collapsed but the haustorium still appears to be active. X 500.

e	embryo
en	coenocytic endosperm
h	haustorium



32

Fig. 33

Micropylar region of an embryo sac with a globular embryo. The suspensor cell is attached to the embryo sac wall. Adjacent to it is the empty remnant of a synergid and its haustorium. The sac is filled with cellular endosperm. X 900.

e	embryo
en	cellular endosperm
h	haustorium (non-functional)
sus	suspensor cell
sy	remains of synergid

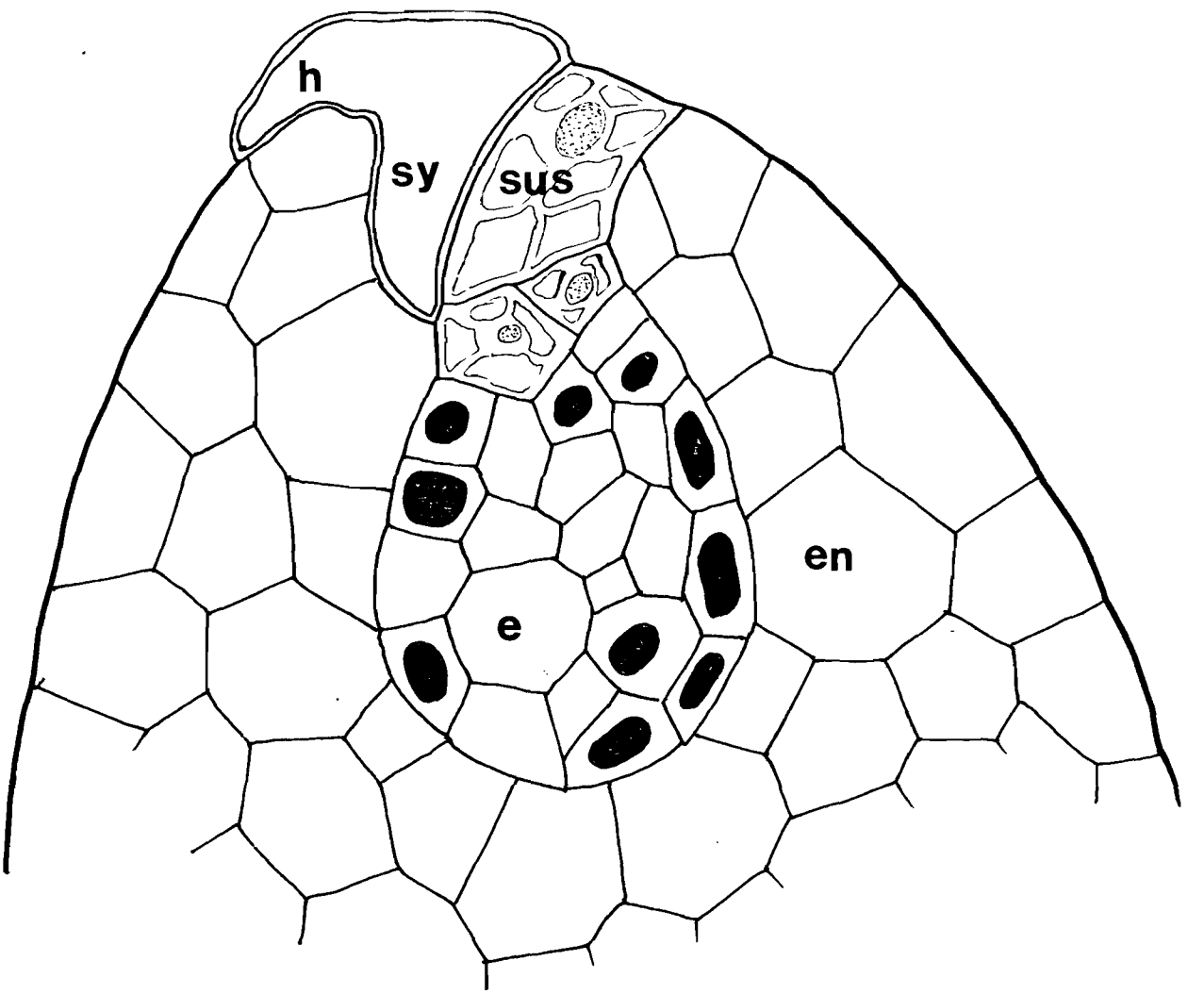

h
sy
sus
en
e

Fig. 34

Globular embryo at a stage similar to Fig. 33. The endosperm is cellular. X 1000.

e	embryo
en	cellular endosperm
h	haustorium (non-functional)
sus	suspensor cell
sy	remains of synergid

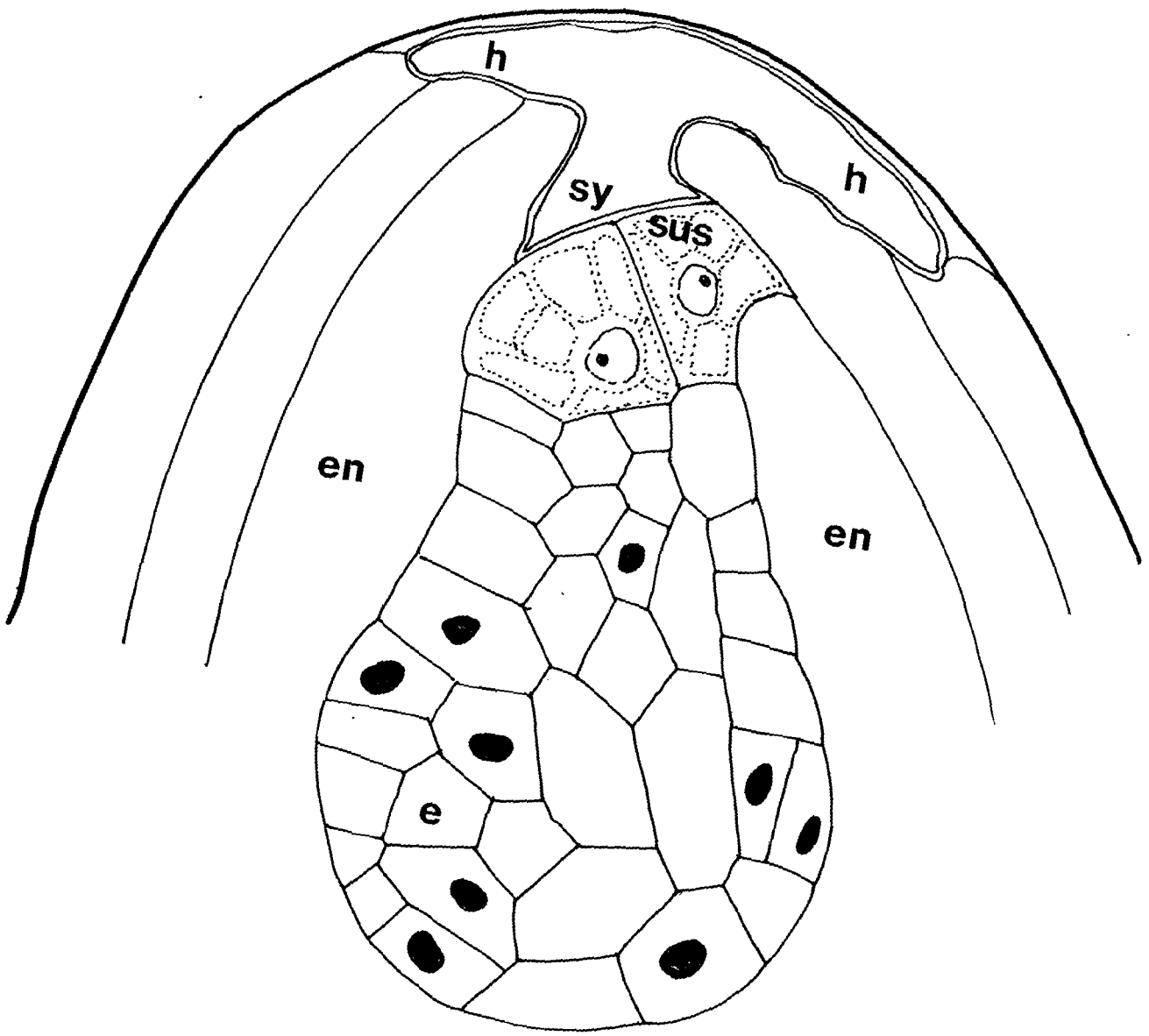

h
sy
sus
h
en
en
e

Fig. 35

LS through an embryo in an advanced globular stage. The embryo sac (which is cut obliquely) is filled with mainly cellular endosperm. The broadly based suspensor is highly vacuolar as are the adjacent cells of the embryo. X 800.

e	embryo
en	cellular endosperm
sus	suspensor

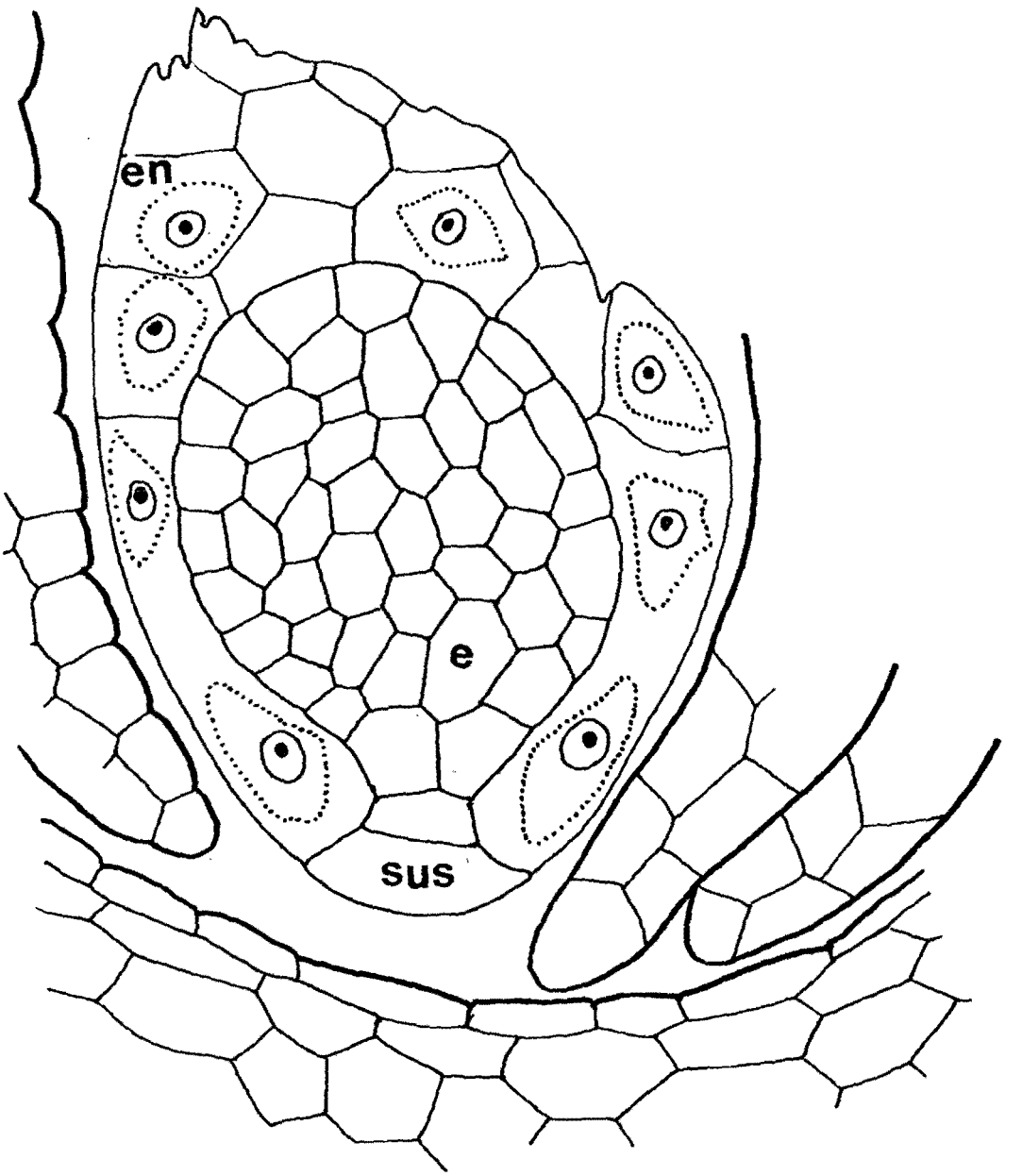

en
e
sus

Fig. 36

Developing caryopsis in LS. X 135.

e embryo

en cellular endosperm filling the caryopsis

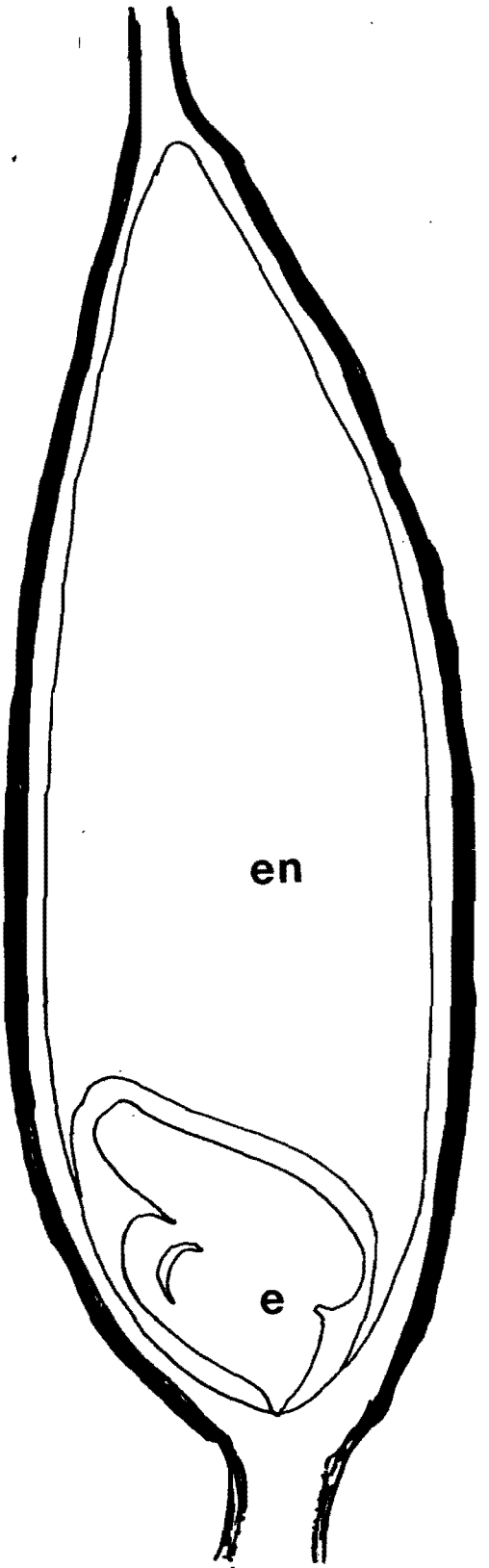
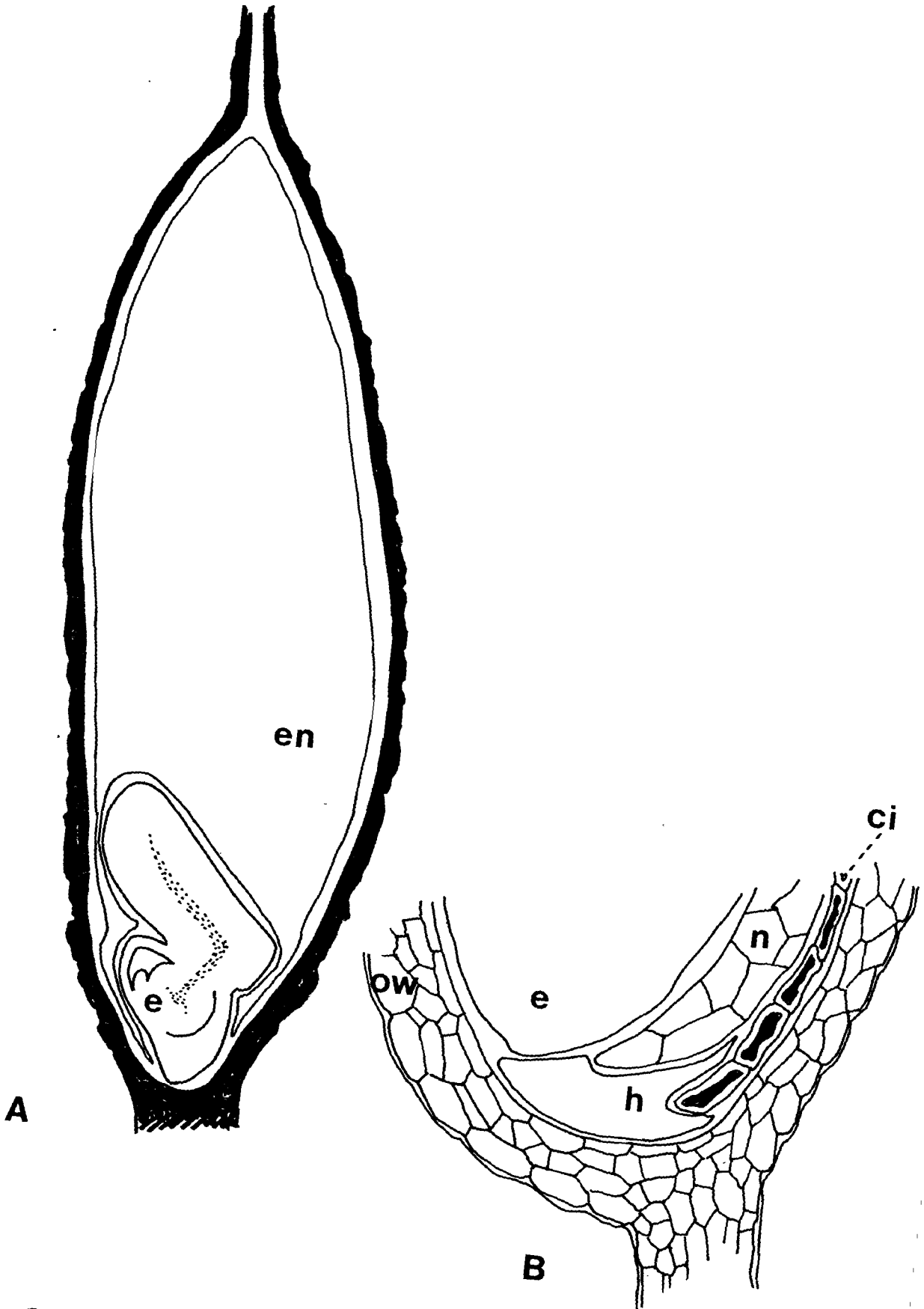

en
e

Fig. 37

Caryopsis in LS. A, developing embryo, with a solid mass of cellular endosperm filling the caryopsis, X 125; B, detail of the base of the caryopsis, showing persistent crushed remains of the haustorium, X 500.

ci	crushed integument
e	embryo
en	endosperm
h	empty remains of haustorium
n	nucellar tissue
ow	ovary wall



37

Fig. 38

Caryopsis in LS. A, advanced stage of embryo, X 165; B, detail of outer endosperm cells and caryopsis wall, X 960.

a	aleurone layer
ci	crushed integument
co	coleoptile
cr	coleorhiza
en	endosperm
ow	ovary wall
sc	scutellum

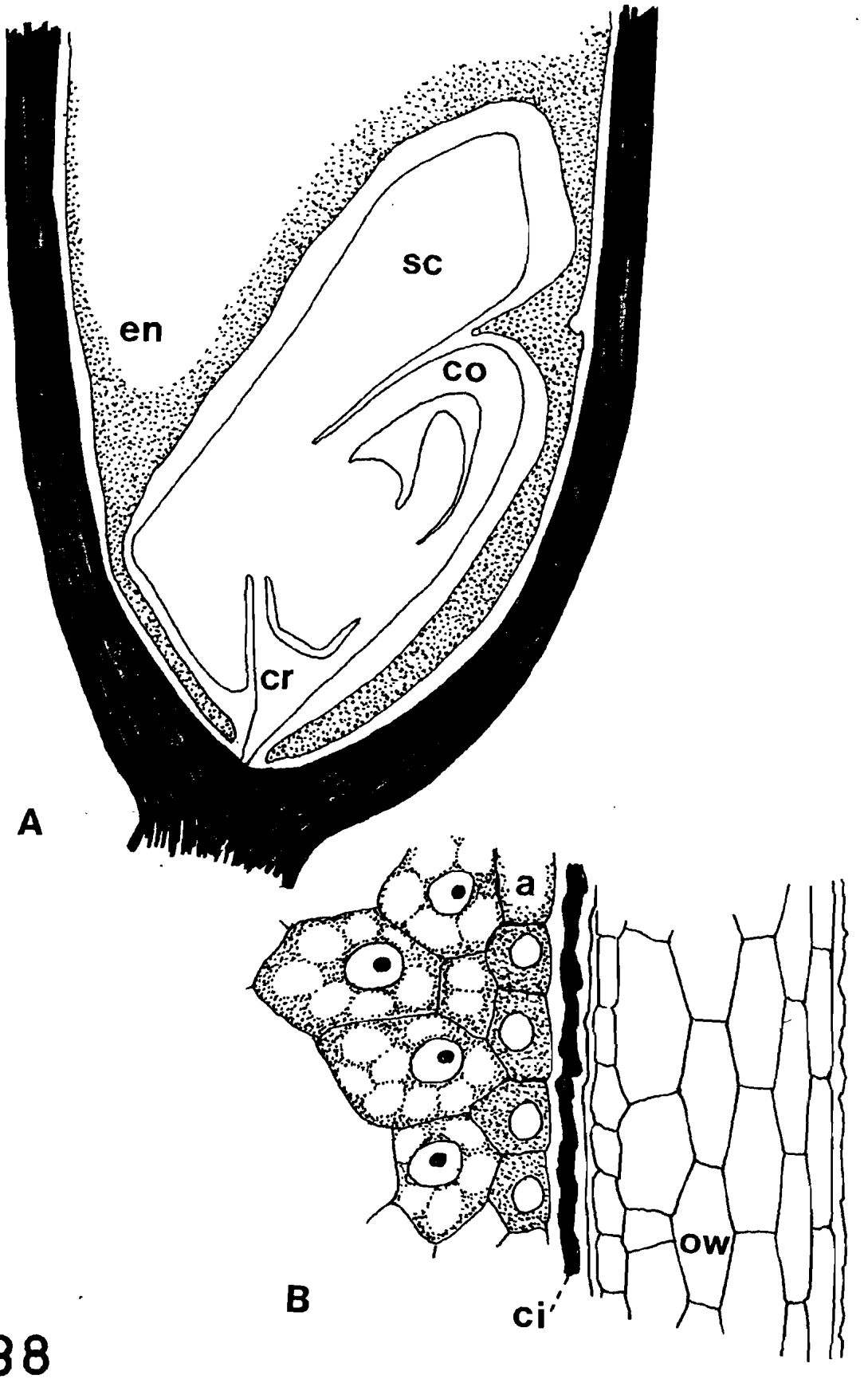

sc
en
co
cr
A
a
B
ci
ow

Fig. 39

Two globular embryos lying together in a mass of cellular endosperm which completely fills the developing caryopsis. X. 100.

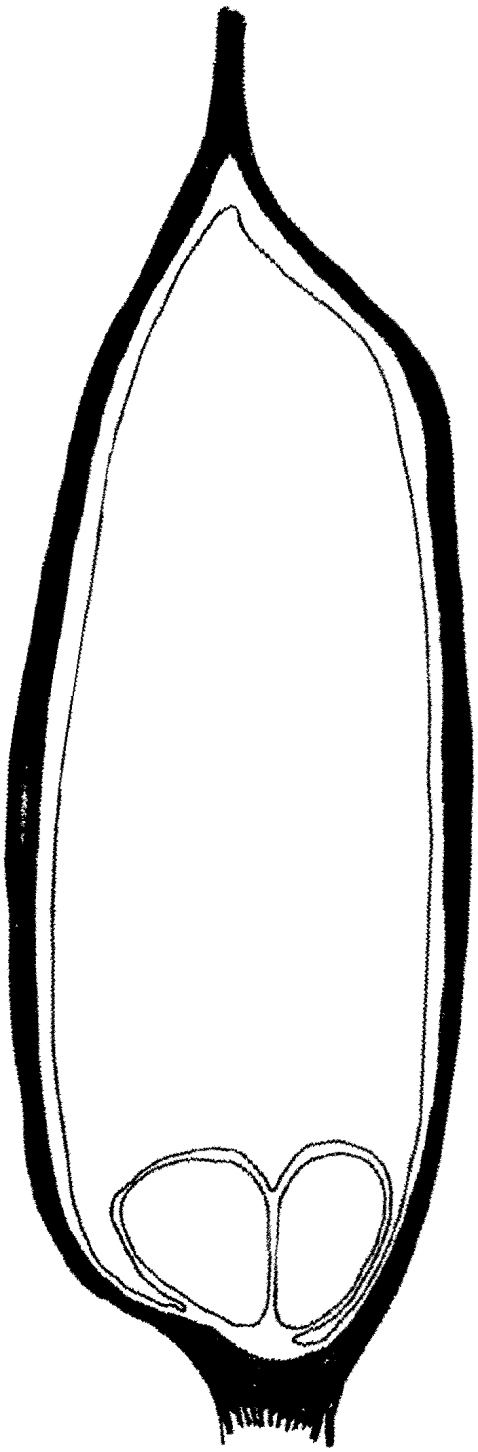
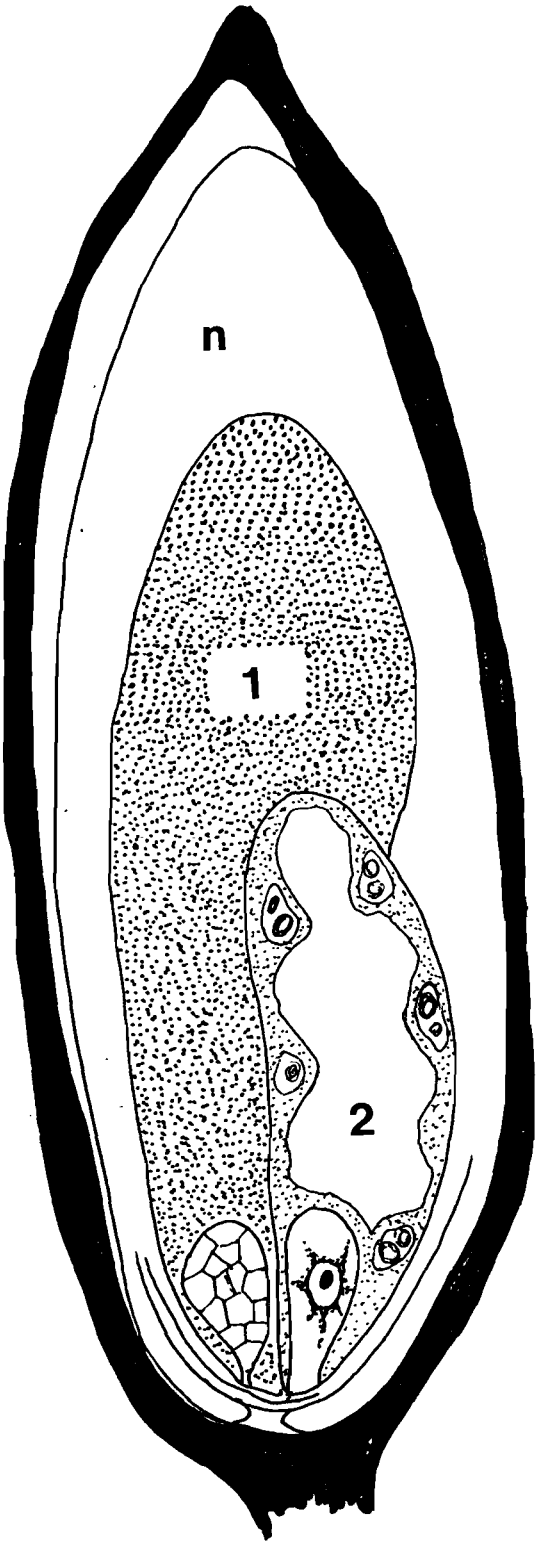


Fig. 40

Developing caryopsis in LS. Two embryo sacs are present. 1, contains a globular embryo and cellular endosperm; 2, contains an undivided egg cell and peripheral coenocytic endosperm. X 165.

n remains of nucellus

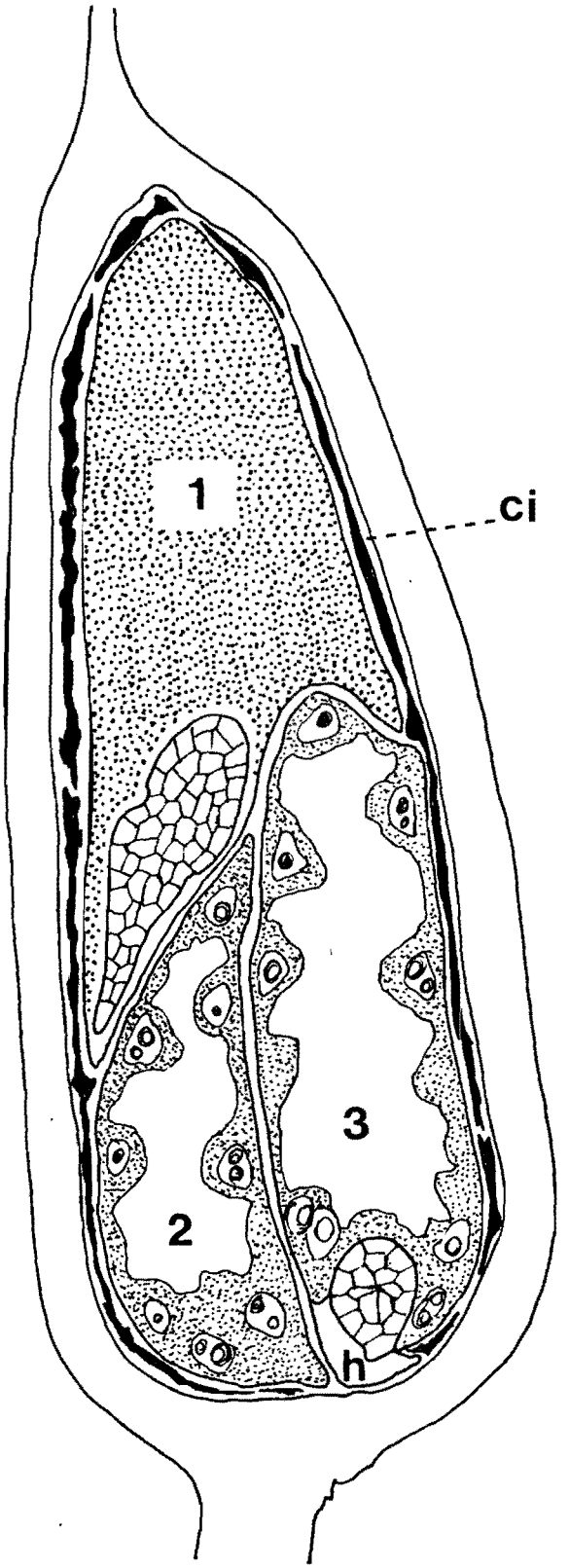


40

Fig. 41

Developing caryopsis in LS. Three embryo sacs are present. 1, contains an embryo and cellular endosperm; 2, contains no embryo but has developed coenocytic endosperm; 3, contains a globular embryo and coenocytic endosperm. The empty remains of a synergid and haustorium can be seen beside the embryo. X 165.

ci crushed integuments
h remains of haustorium and synergid



41

Fig. 42

LS of ovule of a sexually reproducing species, *C. toetoe*. The three micropylar spores of a tetrad have degenerated leaving the functional chalazal spore. X 480.

ds	disintegrated spores
fs	functional spore
ii	inner integument
n	nucellus
oi	outer integument

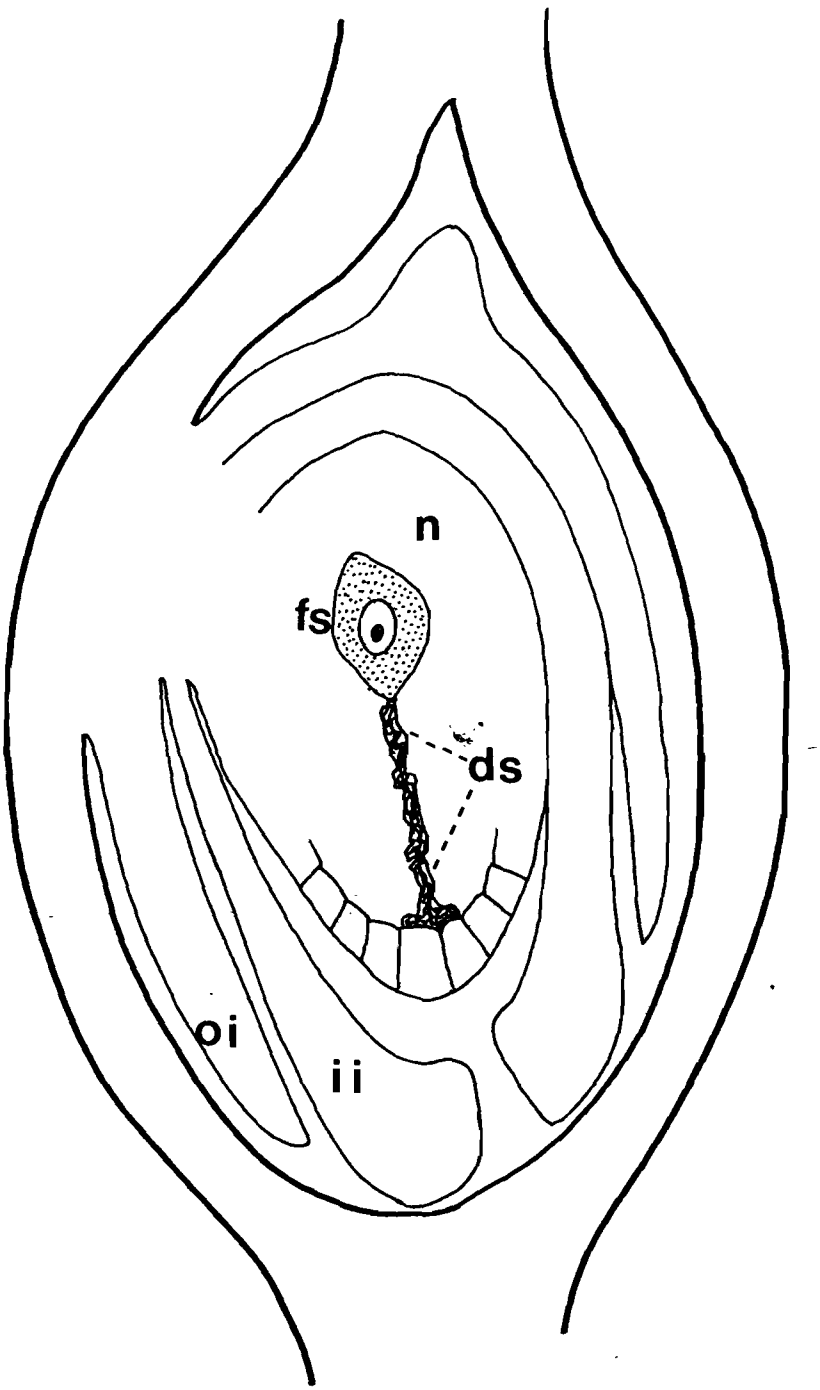

n
fs
ds
oi
ii

Fig. 43

LS of ovule of a sexually reproducing species, *C. toetoe*. The mature embryo sac has a group of antipodals in a rounded caecum at the chalazal end, a central cell nucleus adjacent to the egg cell and two synergids. Each synergid is prolonged as a micropylar haustorium. X 300.

an	antipodal cells
cn	central cell nucleus
eg	egg cell
h	haustorium
i	inner and outer integuments
n	nucellus
sy	synergid

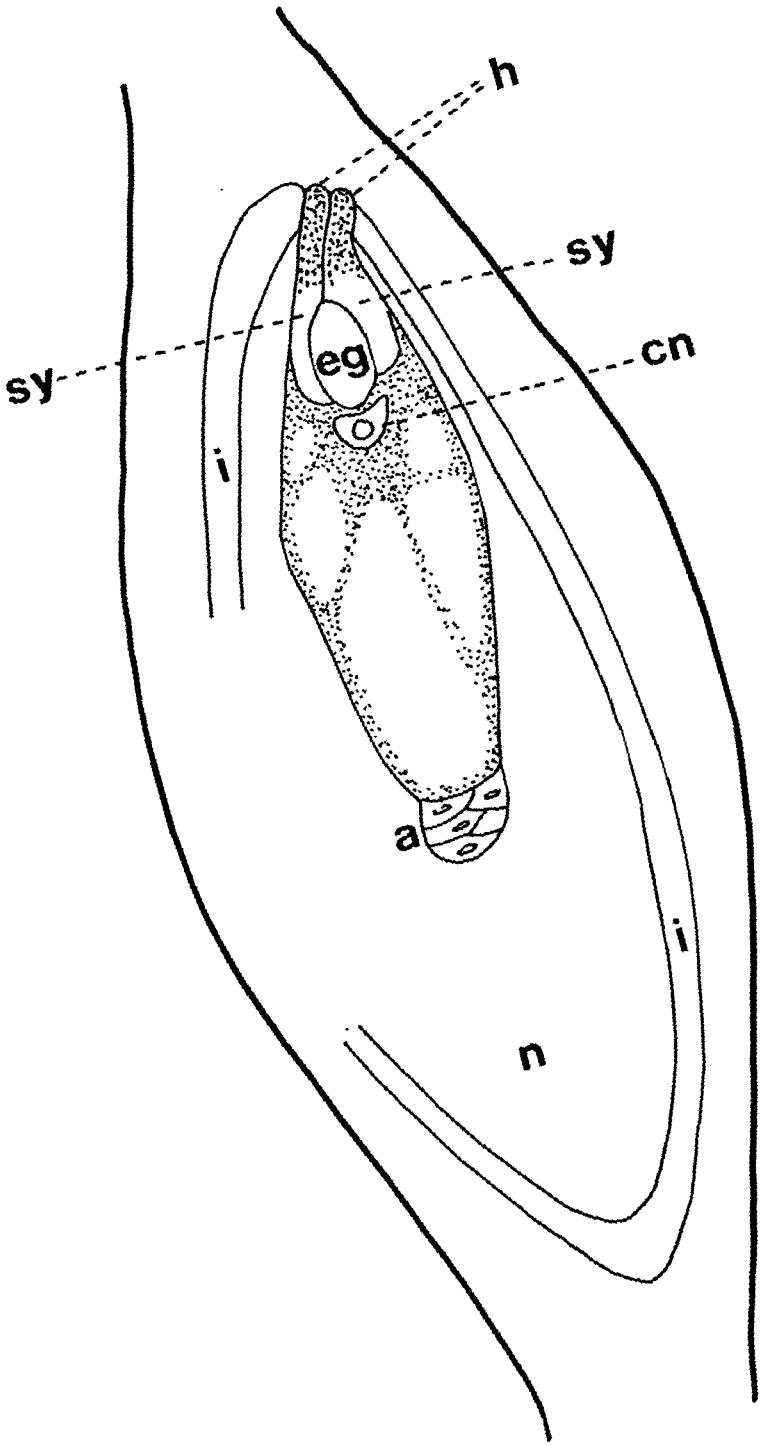
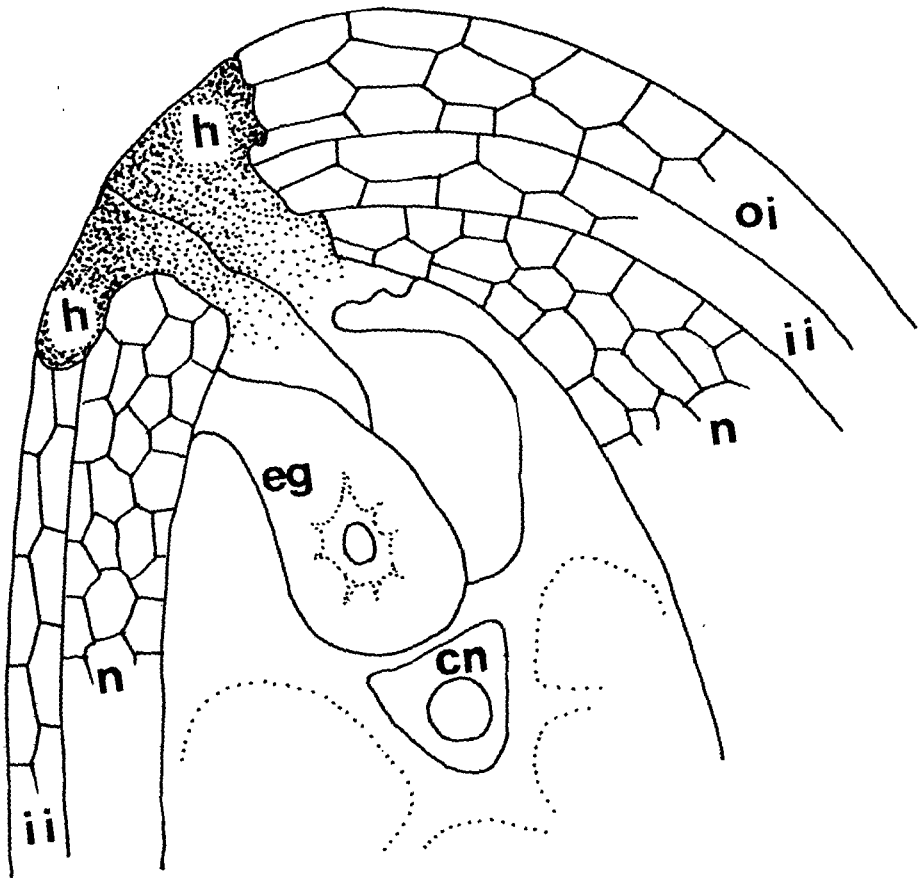

h
sy
sy
cn
eg
i
a
i
n

Fig. 44

Detail of the micropylar region of the embryo sac of *C. toetoe*. The egg cell partly obscures one of the synergids. An haustorium from each synergid passes through the micropyle. X 900.

cn	central cell nucleus
eg	egg cell
h	haustorium
ii	inner integument
n	nucellus
oi	outer integument


h
h
oi
ii
n
eg
cn
n
ii